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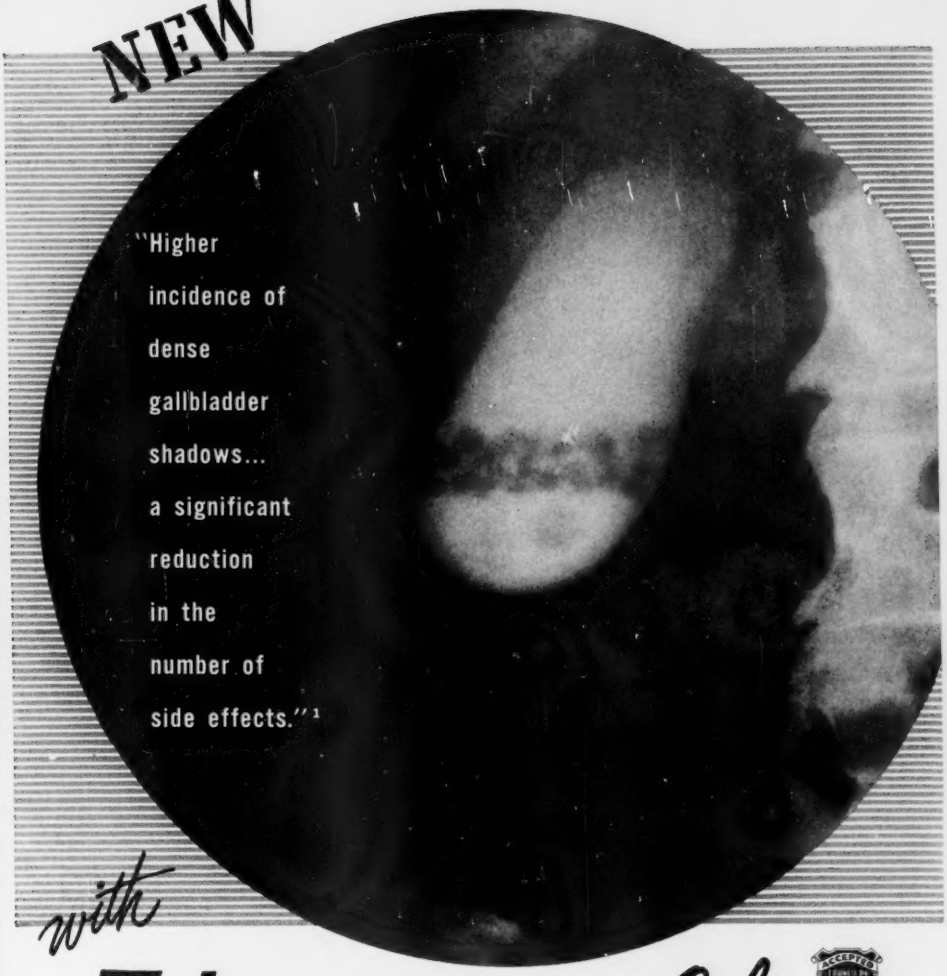

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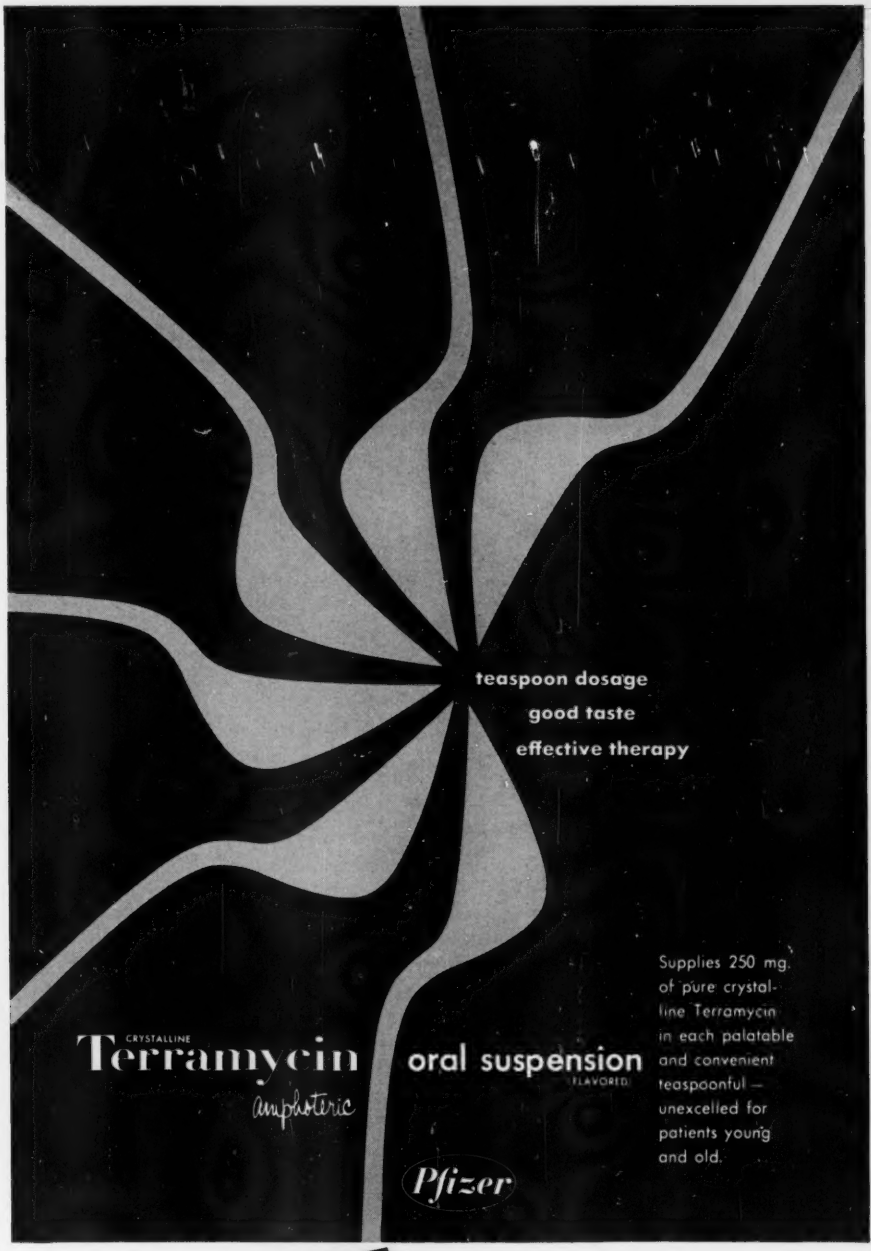

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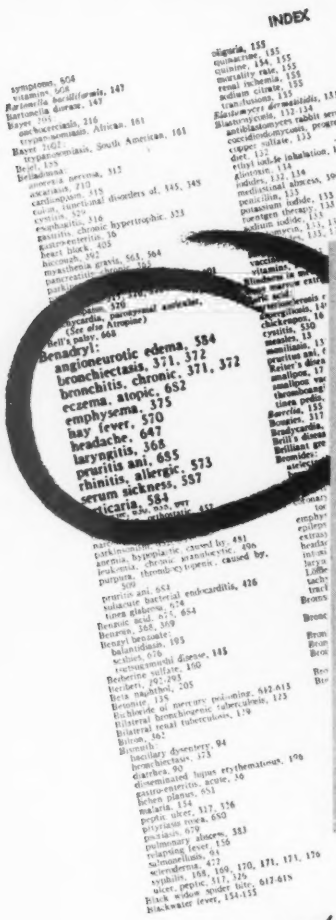

INDEX* *to allergy relief*

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OLFACTORY-LIKE RESPONSES IN PYRIFORM AREA TO NON-OLFACTORY STIMULATION*

PAUL D. MacLEAN, NORMAN H. HORWITZ,** AND FRANKLIN ROBINSON

Considerable doubt continues to exist about the rôle of finely myelinated and unmyelinated† visceral afferents in the function of the cerebral cortex. Recent evoked potential studies involving stimulation of the chorda tympani,[20, 21] the vagus,[6, 7, 14] and the splanchnic nerves[3] have failed to establish that impulses transmitted along the *slow-conducting*‡ components of these nerves reach the cortex. It is inferred, however, from clinical evidence that the slowly conducted signals are discriminated at the cortical level.

The inability to resolve the foregoing problem by the usual physiological techniques indicated the need for other methods. For this reason it was decided to re-explore the bioelectrical properties of the olfactory system in the hope of gaining a clue to the physiology of visceral systems in general. Olfaction is unique in being both an extero- and visceroceptive sense. Its anatomical substratum has attributes that make it a prototype of a primitive visceral system. The primary olfactory neurone manifests a most primitive characteristic insofar as it assumes a position in the dermal layer. The olfactory nerves going to the bulb are entirely unmyelinated. From the primary cell station in the bulb a large proportion of fibers travel in the olfactory tract directly to a phylogenetically ancient part of the cortex. In short, one has in the olfactory apparatus a system that begins with entirely unmyelinated fibers and is irrefutably known to reach the cerebral cortex.

The cortical responses in the pyriform area following natural olfactory stimulation are strikingly dissimilar to those obtained from the neocortex by natural activation of visual, auditory, or somesthetic receptors. In contrast to the latter, one finds an olfactory stimulus is followed by a sustained train of rhythmically recurring sinusoidal potentials of high voltage. Adrian, employing the hedgehog and the cat, appears to have been the first to describe such potentials as occurring in both the olfactory bulb and the pyriform cortex.[1] Figure 1 shows responses to olfactory stimulation recorded from the pyriform area in the opossum, rabbit, cat, and the squirrel

* From the Department of Psychiatry and Laboratory of Physiology, Yale University School of Medicine. This investigation was supported by a research grant from The National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service. Presented at the meeting of the Eastern Association of Electroencephalographers, Johns Hopkins Hospital, Baltimore, Maryland, October 18, 1952.

** Formerly Fellow in Neurosurgery, Yale University School of Medicine.

† Used in the classical sense.

‡ As opposed to the faster conducting components.

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monkey. In view of the similarity of the microanatomical organization of the pyriform cortex in all osmatic mammals,[22] the kind of responses illustrated in the figure might be expected to be found in all such forms.

The rationale for further study of the olfactory system made it particularly significant to perform the following observations: (i) to compare the type of responses obtained, respectively, by natural and by electrical stimulation; (ii) to study the effect of these two types of stimulation on the transynaptic transmission of impulses; and (iii) to ascertain to what extent various anesthetics, convulsants, autonomic drugs, etc., affect the capability

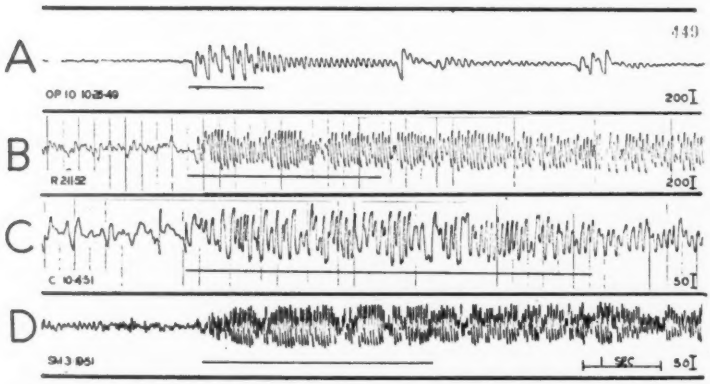

FIG. 1. Responses induced in pyriform area by olfactory stimulation in animals of various species under different anesthetics. Lines under responses indicate approximate duration of current of smoke-filled air through nose. A. Opossum (dial) ([18]). B. Rabbit (nembutal). C. Cat (ether). D. Squirrel monkey (amytal). Calibrations in this and all subsequent figures are in microvolts.

of eliciting responses as well as modify their form and magnitude. In addition, one would look for the influence of other sensory systems on the bioelectrical activity of the olfactory system. During the course of initial experiments employing natural forms of stimulation, it was found that the application of sapid substances to the tongue, as well as hard pinching of various parts of the body, resulted in olfactory-like responses in the pyriform area. These responses, together with some of the significant findings in experiments employing natural olfactory stimulation, are the subject of this paper.

MATERIALS AND METHODS

For these preliminary experiments 30 rabbits, six cats, and two monkeys were used. Anesthetics employed to date have included ether, nembutal, dial, and amytal. In a few instances the isolated brain preparation of Bremer has been resorted to in order to avoid continued anesthesia. Gaining exposure of the pyriform area required the

removal of the temporal muscles, zygoma, and the orbit. The cortex of the lateral convexity above the rhinal fissure was also exposed for the application of electrodes. As yet, the medial surface of the hemisphere has been explored in only one preparation. In those experiments requiring simultaneous examination of the pyriform area and hippocampus, the latter was usually approached through a limited exposure of the opposite hemisphere. In every instance the animal breathed through a tracheal cannula. An olfactory stimulant was delivered through a nasal catheter. In some experiments (not reported here) a variety of gases were administered at various temperatures at a rate controlled by a flow-meter. Solutions of sapid substances (salt, quinine, etc.) were usually applied to the tongue in various concentrations by means of a syringe or a cotton patty, but sometimes undissolved substances were coated on the tongue. Pinching the body wall was performed manually with a surgical clamp. Other forms of sensory stimulation, as well as experimental procedures to be dealt with more fully elsewhere, will be referred to in the appropriate context.

An eight-channel inkwriting electroencephalograph (Grass) was used because of the need to show events that were relatively slow in duration and of long temporal sequence, as well as to compare simultaneously the bioelectrical activity in several regions. The activity in two channels was also viewed on a cathode-ray oscillograph. Electrodes were of small stainless steel wire with ball tips. A reference electrode was placed on the scalp. "Monopolar" recording was used for purposes of exploring, and bipolar recording for localization. All recordings were made in a shielded room with the animal in a headholder.

Results

1. *Observations on natural olfactory stimulation.* As Adrian has emphasized,[1, 2] the character of responses induced by natural olfactory stimulation will vary according to the type and depth of anesthesia. The rabbit anesthetized with nembutal provides a particularly good preparation for demonstrating olfactory responses in the pyriform area and for observing their modification during progressively lighter stages of narcosis. With intravenous nembutal this animal may pass from a very deep to a moderately light stage of anesthesia within a period of an hour. In our experience, in very deep anesthesia (electrocorticogram practically flat) it has not been possible, as might be expected, to elicit any response. After several minutes, however, the blowing of room air into the nose will result in the appearance of a train of rhythmically recurring potentials rising abruptly from a relatively smooth base line. This is illustrated by the top recording in Figure 2 where there is a three-second train of potentials that recur quite rhythmically at about 12 per second. Thereafter, as anesthesia becomes less deep, an induced train of such potentials does not dissipate into a relatively smooth base line, but leaves in its wake a continuous background of random activity of the kind seen at the beginning of line 2 in Figure 2, and which possibly corresponds to what Adrian has termed the "intrinsic activity" in the olfactory bulb.[2] It was as though the stimulus had the effect of arousing the animal out of the deep stage of anesthesia. This random activity, which becomes more prominent in the change from moderately deep to moderately light anesthesia, may at times have intermixed with it potentials that almost have the regularity and amplitude of olfactory-induced responses.

As the animal passes from moderately deep to moderately light anesthesia, it is apparent that the frequency of the induced potentials becomes somewhat faster. This is illustrated in lines 2 and 3 in Figure 2 which shows a recording after the animal mentioned above went into the interim stage. The train begins with potentials having a frequency of about 15 per second, and, as the response continues to outlast the duration of the stimulus, the frequency slows to about 13 per second. As the animal becomes still lighter, one may expect to find induced potentials with frequencies around 20 per

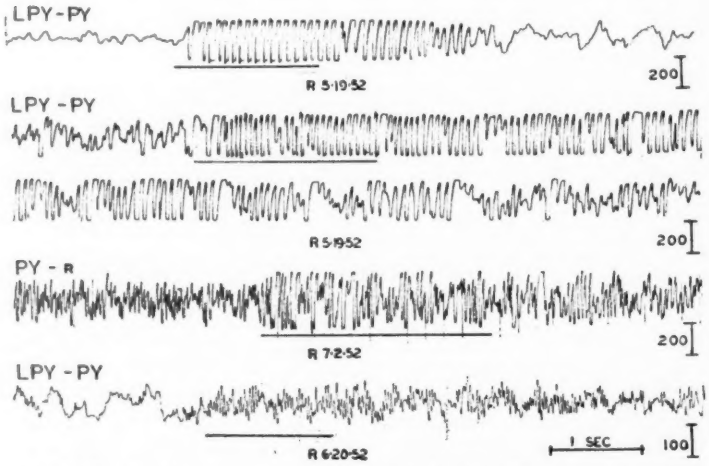

FIG. 2. Samples of responses to olfactory stimulation in pyriform area of rabbit as they appear at various stages from deep anesthesia to wakefulness. Lines under recordings indicate approximate duration of flow of room air through ipsilateral nostril. First line: response in deep nembutal anesthesia. Second line and its continuation in the third: response in the same rabbit typifying the activity seen during the period when animals of this species pass over from deep into moderately light nembutal anesthesia. Fourth line: response in moderately light nembutal anesthesia. Note how the induced potentials are obscured by the spontaneous activity. Fifth line: response in a Bremer preparation after the animal wakened from anesthesia. All samples, except the third, obtained by bipolar recording.

second. Intense forms of stimulation such as smoke (which it must be remembered is a trigeminal as well as a powerful olfactory stimulant) may be accompanied by a train of still faster, rhythmically recurring potentials lasting, with sustained voltage, for many seconds after the administration of the stimulus.

In line 4 of Figure 2 it is seen that by the time an animal has passed into a moderately light stage of anesthesia the spontaneous activity of the pyriform area is so fast and of such great amplitude that it is difficult to demonstrate the presence and the character of the induced response. On the other hand, in Bremer preparations, after awakening from anesthesia has

occurred, we have observed induced responses characterized by fast potentials, at around 35 per second that rise gradually above base line activity.

Our experiences to date with other forms of anesthesia have brought to light no findings that add anything significant to what has already been reported by Adrian. In summarizing the foregoing account one can say that responses induced by natural olfactory stimulation are seen in clearest detail in the period between moderately deep and moderately light anesthesia. The induced potentials show some increase in frequency as anesthesia lightens, obtaining their maximum frequency after the animal has awakened from anesthesia. In good preparations the amplitude of the responses during anesthesia commonly ranges between 400 and 500 microvolts.

The pyriform cortex is a secondary olfactory station. Localization studies in the rabbit indicate that the type of responses described can also be obtained, but with less amplitude, from the cortex (seen in a lateral exposure) just above the rhinal fissure. We have not yet explored the posterior extension of the hippocampal gyrus in these studies. As Adrian has pointed out, the response occurs predominantly ipsilateral to the stimulus.[1] From the work of MacLean and Delgado[16] it is also known that the amygdala, which is likewise a secondary olfactory station, shows similar responses.

In view of the rationale for pursuing study on the olfactory system, it was most important to ascertain whether or not natural olfactory stimulation would activate structures more than two synapses removed from the receptor organ. All evidence to date indicates that the hippocampus receives no contribution directly from the olfactory bulb. If this is correct, it would be at best a tertiary station. Figure 3 shows that instillation of smoke into the *ipsilateral* nostril will bring about synchronized activity in this structure. In *A* of Figure 3, two responses to such stimulation are shown in recordings from a cat that was lightly anesthetized with ether. The employment of natural olfactory stimulation makes it impossible to assess with any exactitude the latency of responses, but our observations gave the impression that the hippocampus responded in a less predictable manner and after a longer time than the pyriform area. In *B* of Figure 3 a response is shown in a cat on which a bilateral removal of the neocortex had been performed and which was then allowed to come out of anesthesia. With nembutal anesthesia, as illustrated in Figure 3C, trains of diphasic spikes occurring at around six per second have been observed to follow olfactory stimulation. The foregoing findings, therefore, suggest that natural olfactory stimulation will activate structures more than two synapses removed from the receptors.

2. *Observations on natural gustatory stimulation.* A number of rabbits were anesthetized by ether in an attempt to duplicate studies reporting that gustatory stimulation resulted in identifiable responses in the masticatory area.[10] No success was achieved by this method. In experiments

employing nembutal anesthesia, however, it was found that application of sapid substances to the tongue resulted in olfactory-like responses in the pyriform area provided that testing was performed at a critical level of anesthesia. If the animal was quite deep in anesthesia, one might get good olfactory responses, but not gustatory ones. On the other hand, as the animal neared a moderately light stage of anesthesia, a similar situation appeared to apply. The gustatory responses were not of as great amplitude as those induced by olfactory stimulation or of as long duration. It is possible, therefore, that the increased amount of "intrinsic activity," as the anesthesia lightened, might obscure them more readily than the olfactory-induced potentials. Lines 2 and 3 in Figure 2 illustrate the character of the

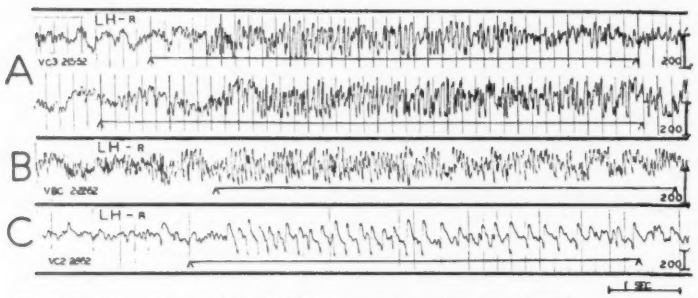

FIG. 3. Responses induced in the hippocampus by instillation of smoke through ipsilateral nostril. Lines under recordings indicate approximate time that smoke was administered. A. Two responses in cat lightly anesthetized with ether. B. Response in cat where the neopallium was removed and the animal allowed to come out of anesthesia. C. An evoked train of diphasic spikes in a cat under nembutal anesthesia. LH – R, signifies "monopolar" recording from left hippocampus.

electrocorticogram at about the time when one has the best success in eliciting gustatory responses.

Figure 4 gives samples of simultaneous recordings from the left pyriform cortex and the right hippocampus in the rabbit. In *A* a typical response to the instillation of room air in the left nostril is given for comparison with the gustatory responses shown in *B*, *C*, and *D*. In *B* a train of rather similar potentials, but of less amplitude and of shorter duration, follows in a little over two seconds the application of salt crystals to the tongue. In the time between *B* and the obtaining of the subsequent records the animal received sufficient tubocurarine to result in seizure activity of the cortex.* This

* Tubocurarine in large doses has attributes of a convulsant drug in regard to its effect on the activity of nerve cells. Convulsant drugs were employed in a number of experiments on the supposition that they might enhance evoked responses in the pyriform area. Tubocurarine holds the advantage over convulsant drugs insofar as it assures muscular immobilization of the animal at the time it is achieving the effects of a convulsant on the cortex. This subject will be dealt with more extensively elsewhere.

activity obscured any evoked responses that might possibly have been present and it had to be abated by intravenous nembutal. After the foregoing medication the response shown in line *C* followed the application to the tongue of a cotton patty soaked in a concentrated salt solution. This was

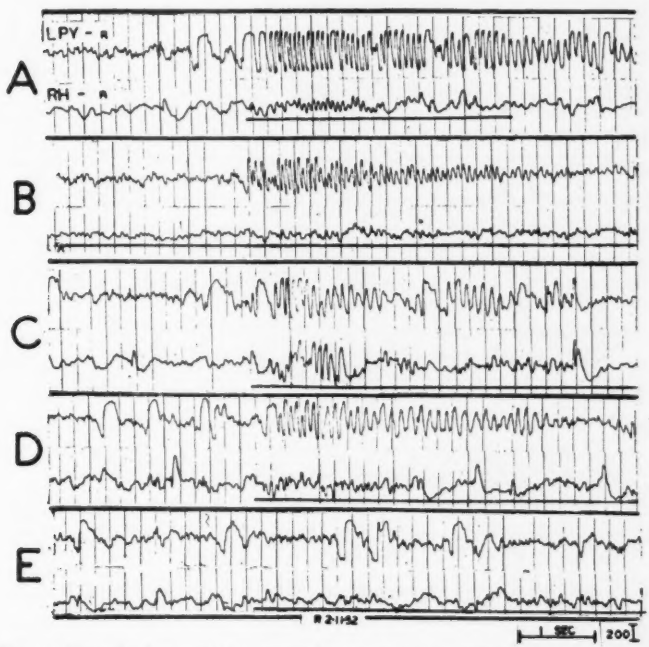

FIG. 4. Records from rabbit contrasting a typical olfactory response (A) with those obtained by gustatory stimulation (B, C, D). Moderately deep nembutal anesthesia. Simultaneous "monopolar" recording from left pyriform cortex (LPY – R) and right hippocampus (RH – R). Lines under recording from hippocampus indicate duration of the respective stimuli. In B a response follows by a little over two seconds the application of salt crystals to the tongue. C shows a response to the application to the tongue of cotton patty soaked in saline. See text for details regarding the changed experimental conditions under which this and the subsequent records were obtained, as well as for a discussion of the hippocampal activity. D, another of several responses elicited by the same procedure as in C. E, failure of response after placing a dry cotton pack in the mouth. The repeatedly negative findings following this manoeuvre indicated the above responses were not the result of touch or light pressure.

one of the two instances in the present experiments where gustatory stimulation appears to have activated the hippocampus as well. The other was in a cat (referred to above) in which the neocortex was removed and the animal allowed to come out of anesthesia. *D* is included to illustrate that the response in the pyriform area could be obtained on repeated stimulation of the tongue, although activation of the hippocampus never again became so

apparent as in *C*. In *E* a large pack of dry cotton was placed in the mouth without evoking any response. This manoeuvre was done in an effort to show that the evoked response did not result from pressure upon the tongue from the cotton soaked with sapid solution. The same procedure resulted in negative findings in other experiments. Other types of control will be referred to below.

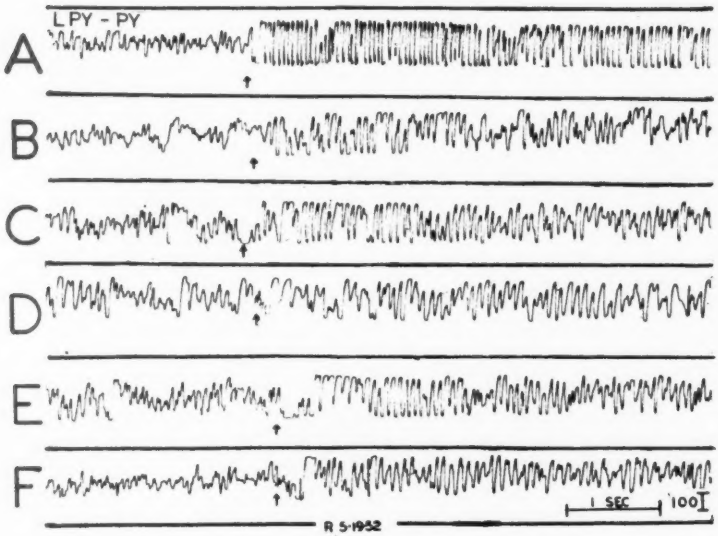

FIG. 5. Bipolar recordings from pyriform area in rabbit showing responses to gustatory stimulation and to pinching the ipsilateral ear. A typical response to olfactory stimulation is shown in A for comparison. This animal was at a somewhat lighter level of nembutal anesthesia than the one in the preceding figure. Arrows indicate application of the respective stimuli. B and C show two responses to the application of a salt patty on the tongue. The response in D resulted from syringing salt crystals off the tongue with water. E and F show similar responses obtained by pinching the tip of the ipsilateral ear. See text for results of noxious stimulation of other parts of the body.

B, *C*, and *D* in Figure 5 give further examples of gustatory responses in the pyriform area of a rabbit in a somewhat lighter stage of anesthesia. They may be compared with a typical response to olfactory stimulation in A. It will be noted that the gustatory responses are of lower amplitude, are less sustained, and that the frequency of the potentials is slighter slower. *B* and *C* show two responses to the application of a salt patty on the tongue, whereas in *D* the response followed a syringing with water that dissolved and spread some salt crystals that had been placed on the tongue.

Although syringing sapid solutions such as salt and quinine on the tongue resulted in responses, this method did not produce as consistent results as

applying the solution by means of a cotton patty. Neither method, however, is devoid of the criticism that the responses were the result of activating other than taste receptors of the tongue. The control experiments employing dry cotton packs were partial assurance that light touch and pressure stimulation were not responsible. Showing that the response could be elicited from the anterior part of the tongue after destroying the connections of the fifth nerve would be further evidence that the potentials resulted from gustatory stimulation, although the chorda tympani also has some somesthetic fibers.[5] Attempts at bilateral removal of the Gasserian ganglion or bilateral section of the third division of the fifth nerve were unsuccessful insofar as they led to a deterioration in the quality of the electrocorticogram. Hence, negative results could not be considered conclusive. Further consideration of this problem is given in the following section.

3. *Observations on noxious stimulation.* In an effort to resolve the rôle of gustatory versus somesthetic stimulation in the activation of olfactory-like responses in the pyriform area (cf. previous section), mechanical stimulation was applied to various parts of the body. Tactile stimulation everywhere was without effect. However, if a hard pinch was applied, for example, to the ear, olfactory-like responses such as illustrated in *E* and *F* of Figure 5 were readily elicited. In this instance, the tip of the ipsilateral ear was stimulated. *F* is included to show that the response is reproducible. Similar olfactory-like responses were obtained from pinching the contralateral ear. It is seen that the responses are indistinguishable from those obtained when a gustatory stimulant was used. The same kind of responses were obtained from pinching the tongue or irritating the skin around the nostril. Clearly identifiable, but less conspicuous, responses were elicited by hard pinching of the toes of either forefoot or hindfoot. On the other hand, pinching the skin of the body did not seem to result in any perceptible change of the electrocorticogram. Hard pinching of the tail, interestingly enough, gave responses with almost as much amplitude as those obtained by stimulating regions of the face and ears. This is illustrated in Figure 7 where responses to a prolonged (A) and a short pinch of the tail (B), respectively, are compared with a typical olfactory response (Fig. 6). It is seen that a single quick pinch of the tail gives rise to almost as great and lasting a response as a sustained pinch. This calls to mind one's subjective experience on receiving a single hard pinch. In the case of the tail and extremities, as elsewhere, the surgical clamp could be put in place, and even partially closed, without any response. This indicates that touch or pressure is not sufficient to elicit the responses (at least in the anesthetized animal), but that the stimulus must be noxious. On occasion, manipulation of the tracheal cannula has resulted in olfactory-like responses in the pyriform area. Further study of the effect of noxious stimulation of the viscera is in progress.

It is to be emphasized that in the foregoing observations electrodes were placed in the frontal, insular, parietal, temporal, and occipital regions, as well as in the pyriform area. The localization of the olfactory-like responses induced by gustatory and noxious stimulation fell within the limits of that described for natural olfactory stimulation.*

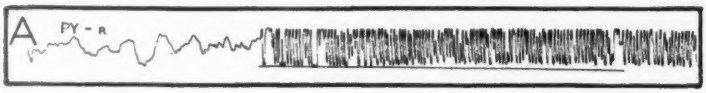

FIG. 6

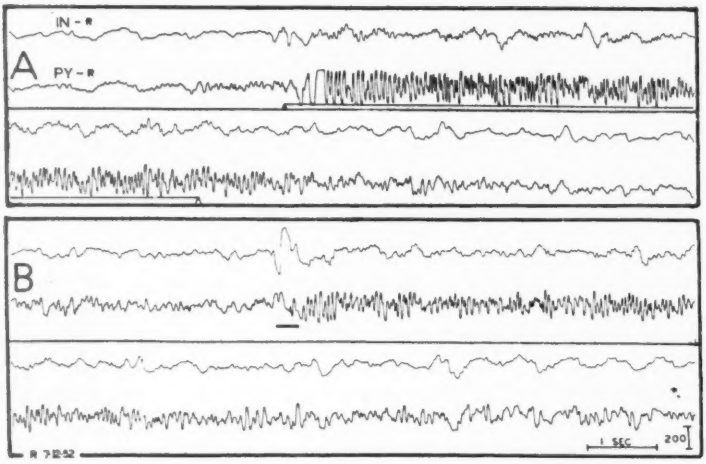

FIG. 7

FIG. 6 and FIG. 7 are recordings from the same rabbit (nembutal anesthesia). FIG. 6 shows a typical response to olfactory stimulation. It is to be compared with the activity induced by a prolonged (FIG. 7A) and brief pinch (FIG. 7B) of the tail. The latter figure shows a simultaneous "monopolar" recording from the insula (IN – R) and pyriform cortex (PY – R). Lines under recordings indicate approximate duration of the stimulus. Note that a brief hard pinch gives rise to almost as great and lasting a response as a sustained pinch.

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* The question obviously arises whether or not the responses described in the previous two sections could be the result of some fortuitous stimulation of the olfactory receptors. It has been our experience time and again that in the animal breathing through a tracheal cannula such a powerful olfactory stimulant as smoke may be enveloped around the head, and even blown into the face, without eliciting any response. This indicates that to elicit a response there must be an appreciable flow of air in the nasal passages. Furthermore, it is to be noted that these responses can be obtained in the curarized animal where the muscular paralysis insures against swallowing setting up any commotion of air in the nasopharynx.

Discussion

This paper has dealt only with observations concerning responses of the kind induced by natural olfactory stimulation. Other types of responses in the pyriform area elicited by auditory stimulation, etc., will be dealt with elsewhere. With one exception, the foregoing observations on gustatory and noxious stimulation have been obtained in the rabbit. It remains to be seen whether or not olfactory-like responses in the pyriform cortex or elsewhere can be elicited in the cat and monkey. In these latter forms, there promises to be greater difficulty in carrying out the experiments at the optimum level of anesthesia.

The pyriform area is part of the limbic cortex surrounding the hilus of the hemisphere. The hilar position of this cortex attests to its early development in phylogeny. Cytoarchitecturally, there are distinctive differences among various portions of the limbic cortex, but taken as a whole, they show similarities that make them easily distinguishable from the surrounding neopallium. In contrast to the latter, they show a relatively poor development of the layers of the outer lamina, particularly those that would correspond to the supragranular layers. In some respects the outer lamina of the limbic cortex is comparable to layer IV of the neocortex because of the distribution of the afferent plexus.[18] The limbic cortex is further distinguished from the neopallium because of its relative paucity of cells of short axis cylinder.[22] These considerations lead one to wonder whether or not activity evoked in other regions of the limbic cortex besides the pyriform area might also show similarities to the olfactory-induced responses. The posterior part of the hippocampal gyrus (angular ganglion of Cajal; entorhinal area of Brodmann) which, as Cajal pointed out,[22] has close affinities with the visual system, is the next obvious place along the limbic ring to test this possibility.

Superficially the induced, rhythmically recurring potentials in the pyriform area have some resemblance to those elicited by the cortical application of acetylcholine, suggesting the possibility of the local release of acetylcholine by nervous stimulation. This problem requires further investigation. But it may be mentioned here that in the rabbit intravenous doses of atropine large enough to lead to seizure activity of the neocortex have the effect of attenuating or even abolishing the induced rhythmic potentials and leaving in their stead a few random spikes. Convulsive drugs such as strychnine and metrazol have a similar effect.

On the other hand, the rhythmically recurring potentials may represent reverberatory activity leading to an after-discharge in closed chains of neurones. It is possible that the relative paucity of cells of short axis cylinder in the pyriform cortex,[22] and indeed the whole limbic cortex, allows reverberatory activity in self-reexciting chains of neurones to take place with greater ease than in the neopallium. It is hypothecated that these cells have a high threshold for excitation "because they generally have only a

few dendrites.'"[12] The more of these elements, therefore, in a closed chain of neurones, the more difficulty there would be in maintaining reverberatory activity. Cajal assumed that the great number of cells with short axons in the neocortex was the anatomical expression of the delicacy of function of the human brain.[cf. 13]

The build-up and long duration of responses in the pyriform cortex following olfactory, gustatory, and noxious ("painful") stimulation suggest a lack of a delicacy of function; and one wonders if there is not manifest in this phylogenetically primitive cortex *one* of the physiological correlates of these sense modalities. All three are senses that give information regarding the intensity and quality of a stimulus, but tell little of its position in space. They are senses too that make it hard to tell subjectively when a stimulus begins and when it ceases. It is also significant to recall that the limbic cortex has recently been found to be implicated in the elaboration of emotion, and emotion is like a visceral sense insofar as it is registered in terms of quality and intensity (for further elaboration cf.[17, 18]).*

It is impossible at the present time, however, to say what significance the observations reported here have in regard to the cortical representation of taste and "pain." One cannot conclude that because stimulation of the receptors associated with these senses activates the pyriform area that the modality of these senses is represented there. Just as speculative would be the inference that these responses are a manifestation of "sensory association" or of an "alerting" or "activation" of the olfactory sense by these other senses. It will be of interest to ascertain whether or not these responses are in any way related to the function of the reticular system.

There is insufficient anatomical knowledge to designate by what route gustatory and somesthetic impressions might reach the pyriform cortex. The work of Morison, Dempsey, and Morison suggests that there may be such a pathway for the somesthetic sense through the subthalamus.[19] There are possible pathways for taste by way of the midline thalamus and the inferior thalamic peduncle, as well as other routes, according to comparative neurologists,[4] through the hypothalamus, medial forebrain bundle, etc.

Finally, it may be recalled that comparative neurologists have long claimed that the pyriform area is an olfacto-somatic correlation center. The present experiments, in addition to pointing to further electrophysiological studies, suggest the desirability of re-examining the work of Ferrier,[5, 9] Horsley and Schäfer,[11] and others in the last century that purported the limbic lobe to be concerned in the appreciation of somesthetic sensation.

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* In the light of this discussion it is also pertinent to recall that individuals with epileptogenic foci involving either the limbic or extra-limbic cortex of the fronto-temporal region are subject to a wide variety of aura that include emotional feelings, olfactory, gustatory, cardiorespiratory, and gastrointestinal sensations, and somesthetic discomforts that range from pains in the head to paresthesias in the buttocks (cf.[18]).

Summary

This paper deals with the first of a series of studies on the olfactory system, the rationale for which is given in the introduction. As already known, natural olfactory stimulation results in a characteristic train of rhythmically recurring sinusoidal potentials in the pyriform area. Such responses, as might have been predicted from the similarity of the micro-anatomical organization of the pyriform cortex in mammals, have now been found in mammals standing low, intermediate, and high in the phylogenetic scale. The present studies have demonstrated that natural olfactory stimulation also leads to a variety of prolonged responses in the hippocampus, a structure inferred to be more than two synapses removed from the olfactory receptors.

In addition, it has been found in rabbits anesthetized with nembutal that gustatory stimulation, as well as the application of noxious stimulation to various parts of the body, leads to responses in the pyriform area that have similarities to those induced by natural olfactory stimulation. The eliciting of the olfactory-like responses appears to be critically conditioned by the level of anesthesia. Possible anatomico-physiological mechanisms responsible for the induced rhythmically recurring potentials and the implications of the present study for further research on the limbic cortex are discussed.

Addendum

Since this paper went to press, one of us has had the pleasure of reviewing the foregoing material with Dr. Richard Jung of Freiburg, and Dr. Jung was reminded of rhythmic responses around 5 to 6 per second that he and Kornmüller obtained from the hippocampus and subiculum when they pinched the hindleg and other parts of the body in the unanesthetized rabbit. The reader is referred to Figure 11 of their paper, "Eine Methodik der Ableitung lokalisierter Potentialschwankungen aus subcorticalen Hirngebieten" (Arch. psychiat., 1938, *109*, 1-30).

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BOVINE SERUM AS A COMPLEMENT FOR THE COMBINED EFFECTS OF THROMBOKINASE AND "CEPHALIN"*†

J. H. MILSTONE

A preceding communication[7] presented detailed evidence that protein thrombokinase and lipoid thromboplastin are distinct factors with complementary functions. It was concluded that thrombokinase is a direct activator of prothrombin, whereas lipoid thromboplastin and ionic calcium are accessory factors. This preliminary note demonstrates that a further complementary effect is exerted by bovine serum.

Prothrombin[7] and thrombokinase[8] were prepared from slaughterhouse bovine plasma, and the general methods were similar to those previously described.[7] The "cephalin" used for the present work was prepared by the classical method of Howell[4]; and it behaved in much the same way as the lipoid thromboplastin used previously.[7] The bovine serum had been stored in a frozen state and had been thawed occasionally for other work.

All tests of Figure 1 contained the same amount of prothrombin and 0.0025 M. calcium chloride. In addition, Test A contained all three variables —0.032 mg. of thrombokinase, 0.016 mg. of "cephalin," and 0.00125 ml. of serum. In Tests B, C, and D, one variable was omitted and the other two were doubled, as outlined in the figure. The final volume of all mixtures was 1.1 ml. At intervals, given by the abscissas, samples were assayed for thrombin by determining how fast they clotted fibrinogen. The arbitrary number, 3,000, was divided by the clotting time in seconds to give the thrombin values represented by the ordinates.

As shown in the figure the net production of thrombin was much faster in the presence of the triple combination—thrombokinase, "cephalin," and serum, than with twice as much of any combination of two out of the three variables. It would be hard to explain the effect of serum as merely that of adding more thrombokinase or more "cephalin." Clearly, the serum exerted a different kind of acceleratory effect. Because of the crudity of whole serum and the complexity of the problem, the distinct effect of serum does not necessarily prove the existence of an entirely distinct accelerator.[8]

But that is a serious possibility, as suggested by further experiments. Net production of thrombin was accelerated by the addition of small amounts of serum to systems which had moderately large amounts of thrombokinase and "cephalin." This revealed that serum brought to the

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system a factor that was not well supplied by all the other materials combined, including the prothrombin and calcium. Similarly, brisk acceleration resulted from the addition of small amounts of "cephalin" to systems containing a large amount of serum. Hence, the serum had very little "cephalin"-like action. The production of thrombin in Test C of Figure 1 was at least partly due to a small amount of thrombokinase which is known to contaminate the prothrombin. How much thrombokinase, if any, was contributed by the serum is unknown; but it could not have been much compared to the amount in 0.032 mg. of the thrombokinase reagent.

When all three variables of Figure 1 were doubled, thrombin was produced faster than in Test A. When the prothrombin was omitted, comparatively large amounts of thrombokinase, "cephalin," and serum failed to produce thrombin. When the calcium chloride was omitted, prothrombin plus thrombokinase produced thrombin as fast without "cephalin" and serum as with them.

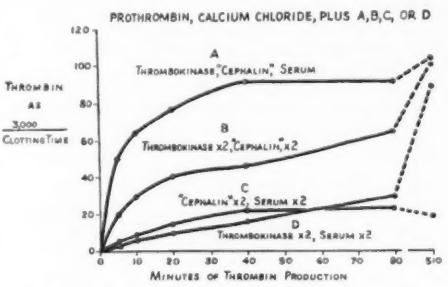

FIG. 1. Bovine serum as a complement for the combined effects of thrombokinase and "cephalin."

Late in 1951 a sample of thrombokinase was sent to Dr. Benjamin Alexander. Several of his results indicated that one or more of the proposed new clotting factors could be distinguished from thrombokinase.[2] Alexander's study gave valuable clues which were followed up in the present work. The results here reported show that bovine serum exerts an acceleratory effect which is different from that of thrombokinase and that of "cephalin." This different effect of serum may possibly correspond to one or more of the proposed new accelerators.

Thrombin-producing systems comprising five separate reagents have been described before.[1, 3, 5, 9, 10] At the moment it is difficult to tell how closely comparable these systems are. The present system differs from all of them in that four of the reagents, rather than three, are named as old factors. It further differs in that one of the reagents, thrombokinase, has been shown to activate prothrombin without calcium ions.[7]

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EXCITATION-CONTRACTION COUPLING IN MUSCULAR RESPONSE*

ALEXANDER SANDOW

When a stimulus is applied to muscle, the response is indicated first by excitation which is set up in the membrane of each reacting fiber, and then by contraction which is a function of the substance within the membrane. If stimulation is effected at a particular spot in a fiber by means of a directly applied cathodal electric shock or by arrival of a nerve impulse at an end-plate, the state of excitation in the form of the self-propagated action potential spreads out in either direction from that spot to the fiber's ends. As recently reviewed by Katz,[45] the evidence is clear that contraction, in contrast, is not itself automatically propagated, but develops locally in each longitudinal segment of the contractile material in consequence of the excitation momentarily present in the nearby membrane as the action potential sweeps by. This dependence of contraction on excitation indicates that there must be some process that is initiated in the excited membrane and which by moving inward makes contact with the contractile elements so as to initiate contraction. We shall designate the entire sequence of reactions —excitation, inward acting link, and activation of contraction—by the term excitation-contraction (E-C) coupling.

Although these general relations of E-C coupling have been known for many years and several attempts[9, 10] have been made to analyze them in further detail, the general concensus among muscle physiologists seems to be that in this sequence the rôle of the action potential and of the link between it and contraction remain very much of a mystery.[45] However, recently there have appeared a number of highly significant findings relevant to this problem. It is the purpose of this article to integrate these results into as specific a pattern of the E-C coupling reactions as is now possible. It will be seen, however, that despite all we have lately learned, there yet remains much to be discovered. Nevertheless, it is hoped that our analysis will serve to indicate such hiatuses in our knowledge and thus stimulate new research in this fundamental problem of muscular activity.

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Mechanical latency and E-C coupling

By their very nature, the events of E-C coupling must occur during the time interval that elapses between the instant of application of a stimulus to the muscle fiber and the instant at which contraction starts. This interval is identical with the mechanical latent period (LP) of contraction and it is therefore evident that studies of the LP should throw some light on the mechanism of E-C coupling.

For nearly a century the LP has been thought of as an interval during which the contractile system is undergoing some transformation which, though free of mechanical change, is concerned with engendering a state capable of manifesting tension at a later moment. In a certain sense this conception is still correct; but modern research demonstrates that even within the usually defined LP, mechanical changes occur. This finding—and others associated with it—are of great importance for our present study since they indicate the earliest moment following stimulation at which the contractile system begins its response, and they therefore serve at least to determine the kinetic inter-relations of the various reactions of E-C coupling. We shall now briefly describe certain aspects of these earliest phases of the contractile response that are relevant to the problem.

It has been, in general, customary to regard the isometric myogram, e.g. of a twitch, as correctly representing the time-course of the tension changes of the contractile units of a muscle. Quite apart from certain complications in such a record due to possible propagative effects (of which we shall have more to say later), Hill[27, 28, 30, 32] has demonstrated by means of quick-stretch experiments that the tension-changes of the basic contractile component, comprising what is called the active state, are quite different from those recorded in the ordinary myogram. The active state is set up very abruptly after excitation, achieving full intensity very early within the conventionally defined contraction period; after a short interval of maintenance it then disappears in a characteristic time course. Furthermore, at full intensity the tension developed, even in a twitch (if maximal), is equal to that of the plateau of the maximal tetanus. Reference to Hill's paper will show how all this information is known; but of interest to us here are the changes that take place during the transition from rest to full activity in the process designated "activation."

Hill's mechanical (quick-stretch) experiments[28, 30, 32] prove that activation —in the sense of the early build-up of tension toward the full active state— is in evidence during the latter half of the LP. He has established further[25, 29, 31] that at this time the excited muscle abruptly begins the liberation of a special thermal output—the activation heat—which is distinct from the immediately following shortening heat that accompanies actual shortening of the activated contractile elements. Furthermore, during this critical period the muscle undergoes a special increase in optical transparency[38] and an increase in pH.[12] And finally it is of interest that Brown,[5] using the tech-

nique of sudden application of high hydrostatic pressure to a muscle, has proved that a reaction (his alpha-process) is already underway during the latter portion of the latent period, which though not directly evident, can nevertheless be shown to determine both the magnitude and the time course of the ensuing contraction. Thus it is clear that during the latter half of the LP a muscle exhibits a number of distinct changes which are indicative of neither its resting nor its active state and which, since they include and are all parallel with the earliest signs of tension onset, must therefore signify some basic alteration of the contractile material that is concerned with the transition between these two states, i.e., with the activation process.

Still another alteration that occurs during the latter half of the LP is the latency relaxation (LR)—a minute, precontractile elongation of the muscle —which was first detected by Rauh[59] by means of a very sensitive optical myograph. His observations have been confirmed and extended by work in our own laboratory[61, 63] which employs the extremely sensitive piezoelectric, cathode-ray oscillographic method. (See Fig. 1.) And Mauro,[54] using a similar technique, has recorded this phenomenon in the responses of single muscle fibers. Our own work and that of Abbott and Ritchie[1] have demonstrated that LR is not an artifact, but that it is an integral feature of the mechanical response of a stimulated muscle; and the work of Mauro proves that this is so for the individual fibers of the muscle.

In a series of papers from this laboratory[61, 62, 63, 72] evidence was presented that the LR is a manifestation of a "tension-induction" process (i.e., what we now call activation) of the contractile material. Recently, however, Hill[32] has contended that the LR does not originate in the basic contractile substance, but that it may be set up in some other structure—the excitatory membrane or the sarcolemma—and that it may appear as a by-product of excitation as such rather than of activation. This problem is discussed at length elsewhere (Sandow[67]), but since we will use the LR in this paper as a sign of activation, this controversial question requires some discussion here. Two points seem to be at issue: is the LR a by-product of excitation or of some activity of the contractile material? And, in what structural component does it occur? Our earlier results[61, 63] proved that the LR may be dissociated from excitation changes.* This then suggests that alterations of the contractile material are responsible for it; and this view is strengthened by the fact that the LR develops not merely, as previously stated, during the general latter part of the latent period, but (as is evident by comparison

* Although in our work the first sign of any mechanical change following stimulation is the latency relaxation, Göpfert and Schaefer[20] using electronic recording methods at extremely high (10^6 x) voltage amplification claim to have registered in frog gastrocnemii a small mechanical change earlier than the LR. This is oppositely directed in respect to the LR and it is supposed to occur in synchrony with the action potential. There may be such an alteration; and if there is, then this would be further evidence that the LR is not a direct result of excitation. But the above authors do not state whether the mechanical and electrical changes were picked up at the same spot on the muscle. And there is the possibility that their pre-LR modification is a stylus artifact like that reported from our laboratory.[63]

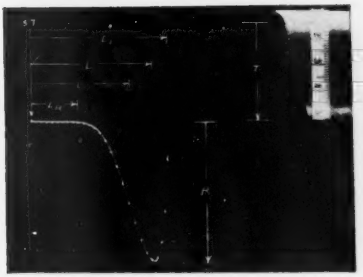

FIG. 1. Typical latent period mechanical response, showing the latency relaxation, of an isometric twitch of a curarized frog sartorius muscle at 13° C. Supermaximal shock, 50 μ sec. in duration; the shock artifact can be seen at the beginning of the sweep, the timing spots on the record occur at 0.2 msec. intervals. The broad bands of light at the upper right are corresponding optical myographic records, the upper indicating the resting tension of the muscle (2 gms.), and the lower marking the deflection due to the developed tension at peak of the twitch (T). On the figure are indications of the time intervals of general interest, all measured from the instant of stimulation: L_R, to the beginning of the latency relaxation; L_0, to the point of inflection of the LR; L_e to the end of the LR; and L_1, to the onset of tension development above the initial tension. (L_1 measures the duration of the latent period as usually defined.) The depth of the LR is symbolized by R.

with the work of Hill[25, 27, 28, 30, 32]) in very close coincidence with the time of onset of the most direct signs of activation of the contractile substance (activation heat, and the initial tension changes revealed by quick-stretch experiments). Hence, we infer that the LR is a by-product of activation.

But whether the LR therefore actually occurs in the contractile material or some other muscle fiber substance (e.g., the sarcolemma) cannot now be unequivocally settled. It is conceivable that it might develop in the contractile elements themselves—and this would signify that some molecular change of these elements causes them to elongate as they become activated. Evidence supporting this view will be found in a previously cited paper.[37] Or it might develop in such a structure as the sarcolemma—this then meaning that some agent emanating from the activation process acts on this material, causing its elements to lengthen and thus be recordable as the LR.

However, for our present needs it is immaterial whether the LR has its direct source in the elongation of structural elements of the contractile component or of some other material of the muscle fiber. Our main point is that the LR is a consequence of, and therefore may be used as a sign of activation; and for this assumption the foregoing discussion of the observed changes during the latter portion of the LP gives clear support. The particular value of utilizing the LR in this fashion becomes evident from the following considerations. Hill made his studies of activation by means of two principal techniques, thermal and mechanical (quick-stretch), both of which, despite remarkable recent improvements, are still not as good in temporal resolving power as is required for general application. The key experiments therefore have been performed at 0° C. at which the muscle responses are so slow that the inherent limitations of the experimental methods no longer stand in the way of obtaining the desired degree of temporal resolution. However, for reasons that will be clear later, we find it necessary to know and to correlate the kinetics of various latent period reactions at temperatures considerably above 0°. For this purpose some signal of activation should be used which can be registered by a method which is fast enough to keep pace with the greater speed of muscle responses at higher temperatures. And this is adequately supplied by the LR, since the electronic method used for recording it is for all practical purposes free of any time lag.

Hence, in responses of a muscle obtained under any conditions we shall use the onset of the LR to mark the instant at which activation begins. Knowledge of this instant has an important bearing on our attempt to elucidate the mechanisms of E-C coupling, for on the basis of present evidence the contractile system does not in any way begin to participate in a response until its activation process is underway. This therefore indicates that during the first half of the latent period (measured by our interval L_R, see Fig. 1) the events associated with excitation and with the link that connects excitation to activation must occur.

Kinetics of E-C coupling reactions

We shall now attempt to make a detailed correlation of the kinetics of the sequence of excitatory and mechanical changes that occur in E-C coupling. A correlation of this sort was made by Fulton[19] a generation ago, but the many advances achieved since then in the study of both excitation and contraction enable us to do this today with greater precision and significance. Yet our present, more sophisticated, knowledge introduces several complications concerning the relative timing of the pertinent events that must be carefully analyzed so that we may build our correlation on a proper basis.

It was shown in the introduction to this paper that contraction of any element of length of the internal contractile substance of a fiber is a local sequel to excitation in the contiguous length of membrane. The ideal temporal analysis of the E-C processes therefore requires that the observable alterations of the relevant systems be recorded at the same point along the length of a muscle. This cannot be accomplished, at least directly, by any methods that introduce propagated disturbances such as occur physiologically in association with the propagation of the muscle impulse or purely mechanically due to transmission of a wave of tension change in the inert elastic structures of the muscle. The desired kind of record of the latency mechanical events can be obtained if the stylus is set transversely across the belly of a muscle and it is arranged to serve as the cathode of a stimulating circuit as well as for mechanical pickup. In this way the latency relaxation is registered as a radial change (a decrease in thickness of the muscle fibers corresponding to the longitudinal elongation of the LR) at the point of initial excitation, thus eliminating any propagative effects along the muscle length.[63, 65] But the simplest and most effective method is massive stimulation of the muscle and axial recording.[68] In this procedure the muscle is fully immersed in Ringer's solution and symmetrically flanked by Ag-AgCl electrodes of large area. Application of a shock to the electrodes results in a 'massive' current through the intervening volume of the bath and so, transversely, through the whole muscle. Earlier work utilizing this method for studies of single muscle fiber responses[8, 73] seemed to indicate that mechanical responses were obtained free of membrane excitation. However, our own work (unpublished) with intact muscle proves that excitation does occur. Although we have not attempted to demonstrate this by relevant electrical recording, we have found that the usual mechanical counterparts of excitation occur, namely, the presence of definite shock strength for both threshold and maximal responses, the absence of increased contractile output for supermaximal shocks, and, especially, the presence of the standard evidence for refractoriness. It may also be mentioned that corroborative evidence for the possibility of massive excitation of muscle fibers is found in the fact that action potentials are produced by nerve fibers when treated

with massive shocks by a method which though different from ours is still, in effect, comparable with it.[36, 53]

Now the particular value of excitation of muscle by massive stimulation lies in the following considerations. In our experiments we have used shocks that are considerably supermaximal, so that any effects due to differences in threshold that may exist from point to point along a given fiber, or among the fibers of the whole muscle, tend to be nullified. Furthermore, the shocks are quite brief—50 to 100 μ sec. Thus, when such a massive shock is applied, the entire face of the surface of each fiber directed toward the cathodal electrode has impressed upon it an adequate cathodal pulse in a short interval of time. Excitation is then set up essentially simultaneously at each element of length of the entire muscle, thus ensuring a mechanical response which, though recorded at an end of the muscle, is unburdened of any propagative complications. And, since the shock is so brief, the zero of time for analysis of this response may be taken, with negligible error, as the instant of application of the shock.

The fact stressed in the foregoing that massive stimulation results in synchronous excitation of the entire length of each fiber means of course that excitation as well as contraction is unpropagated—a point of considerable interest for our main subject, as will be seen later. But the immediate problem is to determine the kinetics of such excitation for correlation with the time course of the corresponding latency mechanical events. This could be done most appropriately by using action potential records obtained under conditions of massive excitation by very brief, supermaximal shocks. Since these are not at hand, we must turn to records of the propagated action potential to give us the necessary information. For this purpose we fortunately have available the highly precise records of membrane action potentials of single muscle fibers recently obtained by Nastuk and Hodgkin[56] and by Fatt and Katz[14] by means of internal microelectrodes.

It must first be noted, however, that the propagated electrical disturbance consists of an initial, slowly rising, and relatively long foot which is followed by the sharp, rapidly varying spike potential. The transition between these is at the point of inflection of the rising curve of the action potential. Now the foot reflects the electrotonic and local response changes—essentially excitatory latency events—and only the spike signifies excitation.[42] It is known that the latency events are quite variable in duration and shape, depending on whether they are recorded at the point of initial excitation (e.g., at an end-plate or at a stimulating cathodal electrode) or after propagation has occurred.[14] In particular, if excitation is caused by a brief, intense shock and the electrical record is registered at the cathodal electrode, the spike appears after a negligible latency and thus initiation of excitation occurs essentially in synchrony with the instant of application of the shock. Indeed, if the shock is of the order of ten times threshold, the work of Hodgkin *et al.*[36] shows that not only the foot but also the entire rising phase

of the spike is completed during the shock interval, and then only the parts of the action potential from crest onward appear on the record. However, barring excitation by such extremely super-threshold stimuli, the spike potential in either nerve or muscle fibers at a given temperature is remarkably invariant despite wide differences in the mode of its elicitation.[14, 88] Such behavior is to be expected, since as soon as a stimulus (whether it is the current delivered by an external generator or the local currents of propagation) changes the membrane potential to the point of instability, the spike potential then runs its course in terms of the inherent electrical properties of the excited membrane. (A strikingly exceptional form of the excitation potential is evoked by transmitter action at the muscle end-plate.[14, 87] But even here, special membrane properties determine the form; and even though the spike potential is lower than normal and is followed by an anomalous "hump," the general kinetics of its rising phase are quite normal.)

From the foregoing considerations it is reasonable to assume that in our massively stimulated, unpropagated muscle responses, a spike potential develops which, though in phase throughout the whole length of each fiber, has the same form as that characteristic of the spike in the propagated disturbance. And furthermore, since the massive shocks we use for evoking mechanical responses are brief and several times maximal strength, there is a negligible foot to the action potential so that the initiation of the spike may be considered to be coincident with the instant of application of the shock. It is thus evident that the course of our massive excitation is derivable from the propagated action potential of muscle fibers if we neglect the foot in such records and equate the moment of initiation of the spike with the instant of massive stimulation.

Fortunately, the work of Nastuk and Hodgkin[58] provides us with precisely measured data obtained over a wide range of temperature on the course of the action potential in fibers of the frog sartorius, which is the same kind of muscle as that which we have used for mechanical recording. These authors do not give explicit data on the duration of the spike, but their Table 3 gives the time required for the action potential to rise from 20% to 100% of crest height. At 20% of crest, the muscle membrane is depolarized only to the extent of about 20 to 25 mV. Bearing in mind that the point of inflection of the rise of the action potential indicates the beginning of the spike, it is clear from Nastuk and Hodgkin's records, as it is also explicitly proved by the results of Fatt and Katz,[14] that this amount of depolarization is only about half that required to discharge the spike. But the next 20 mV. or so of depolarization needed to reach the critical level to initiate excitation is achieved very rapidly. During this phase of activity, the rate of rise of the potential, e.g., at 13 to 17° C., is of the order of several hundred mV. msec.$^{-1}$, and thus the additional depolarization of 20 mV. would develop in only about 0.1 msec. Hence we shall use Nastuk and Hodgkin's temporal values corresponding to the change between 20 and 100% of crest to represent the

time course of excitation of the muscle fiber. It should be realized, however, that such times include a bit of the latter part of the foot and are thus a little too long.

On the basis of the foregoing we now present in Figure 2 the temporal correlation of E-C coupling processes as they occur at 13° C. The course of the spike is derived from the results of Nastuk and Hodgkin and that of the latency mechanical changes from our own average results obtained at the same temperature. The kinetics of the known processes represented in this figure enable us to delineate the sequence of reactions making up E-C coupling. Several of these reactions stand out as clear sign-posts: the rise of the action potential spike, denoting excitation; the latency relaxation, signalling activation; and, at the point of inflection of the L_R, as measured by L_O (see Fig. 1), the earliest sign of tension development (see Sandow[91, 93]), indicating that the active state is being set up and contraction is underway. It is very significant that after excitation is completed—in the sense of the time taken for the spike to achieve its crest (in about 0.6 msec.) — there then occurs an interval of about 1.2 msec. which is still localized within the period of complete mechanical quiescence, i.e., within the interval L_R. This indicates that during the time represented by L_R two processes occur: first excitation and then some other reaction. In the introduction to this paper it was made evident that there must be some process which is initiated by the excitation changes in the membrane and which then acts inwardly to effect activity of the contractile material. We assume that this process is going on during the latter part of the period L_R, for this segment of time—lasting 1.2 msec. at 13° C.—intervenes between the instant at which excitation is complete and the moment at which activation of the contractile material is just detectable. In view of its function, we shall refer to this process as the 'spike-activation (S-A) link.' The speed of action of this link may be measured by the shortness of the time lapse between spike crest and the initiation of the latency relaxation. But it should be noted that other evidence, e.g., the time required to complete activation throughout all of the contractile material, and thus set up the active state at full intensity,[28] indicates that its duration extends into the early part of the usual contraction period.

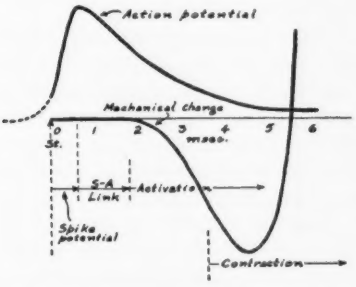

FIG. 2. Temporal correlation of excitation and mechanical events during the latent period of the frog sartorius muscle at 13° C. Only the spike of the action potential is included in the correlation; the neglected foot is indicated by dashed portion of the action potential. See text for details.

Thus, our kinetic analysis of the observable excitatory and mechanical events of the latent period shows that E-C coupling consists of a sequence of four main reactions: (i) spike potential, (ii) S-A link, (iii) activation, and (iv) contraction. To some extent we have already discussed the rôle of activation in this sequence. But of particular interest are those processes which occur in the membrane and the reaction which connects these to the activation of the contractile material; and much remains to be said regarding these functions as performed by the spike potential and the S-A link. We therefore turn to a more detailed discussion of these two reactions.

Currents and potentials during excitation

When a muscle fiber is excited so that propagation occurs—the normal process in the body—we postulate that essentially the same E-C coupling reactions occur as are present when excitation is massive, that is, the arrival of the action potential at a particular segment of the fiber sets in motion the E-C coupling mechanism in that segment. Then, as the action potential sweeps on, this mechanism is repeated in each of the successive longitudinal elements of the fiber so that a dependent wave of contraction follows in the wake of the self-propagated wave of excitation. Under these conditions propagation is determined by the flow of local currents between the resting region just ahead of the spike and the region in the state of excitation.[42] Thus the propagation of excitation includes two rather distinct electrical events, the local currents and the alteration in trans-membrane potential of the excited portion of the membrane. The question therefore arises as to which of these changes serves to initiate the E-C coupling sequence. There is an implication, of course, in our reference to the first event of this sequence as the spike potential that it is the potential change, as such, and not the local current that is of importance. Certain results will now be presented indicating that this implication is correct.

Strong presumptive evidence in favor of the view that local currents play no rôle in E-C coupling has been presented.[17, 18, 21, 46, 47] It is proved that any agent, chemical or electrical, that depolarizes the muscle membrane causes a contracture; and conversely that any other influence which repolarizes causes reversal of the contracture. Kuffler especially has pointed out[46, 47] that a muscle in contracture when completely immersed in a depolarizing solution, e.g., one containing extra K^+ ion, cannot possibly develop local currents since the membrane potential is the same over the entire length of each fiber. The contracture shortening present under such conditions must then be a consequence of the change in membrane potential. Katz,[45] in his short but perspicacious review, comes to the same conclusion. This view, however, is not unequivocal. In the first place, certain muscles, e.g., the frog rectus abdominis, remain in contracture for as long a time as the depolarization is maintained; but other muscles, e.g., the sartorius, develop a contracture during the early period of a depolarization, which then spon-

taneously relaxes even though the depolarization persists.[15, 18] Furthermore, contracture is in many ways different from contraction, especially in timing. In the former there is a general temporal parallelism between shortening and depolarization, while the latter, as indicated in Figure 2 (although the twitch shortening is initiated by the spike potential) runs its course for a long time after the spike is over and thus (except for a small, slowly decaying negative after potential) occurs in the absence of any membrane electrical alteration. It is clear that on this question we need independent evidence obtained from normal contraction behavior; and this evidence is afforded by our experiments with massive stimulation.

As previously discussed, massive stimulation results in excitation without propagation since all longitudinal elements of each fiber are in phase during the action potential response; i.e., there cannot be any potential differences along the length of any fiber undergoing excitation and thus no longitudinal currents can flow. Yet under such circumstances an essentially normal contraction develops. This demonstrates that longitudinal currents are not necessary for E-C coupling as it occurs in response to massive stimulation. But if this is so, then even in propagated responses where such currents occur, there is no reason for believing that they play any rôle in the coupling mechanism. Thus, our considerations make quite conclusive the inference for contractions drawn by Kuffler from his results on contractures. They are furthermore in accord with the view of Katz[45] that when current flows through a muscle fiber, no alterations in ionic distribution occur in the fiber interior, e.g., accumulation of ions at the interfaces between myofibrils and sarcoplasm; for in the absence of such ionic changes there would seem to be no physical basis for longitudinal current effecting activation of the contractile substance. And this applies to any transverse component of current, as well as longitudinal, as long as the current flows in the myoplasm.*

We now consider the electrical changes of excitation which occur transversely across the membrane, and it will be seen that these are of critical importance. However, it will help in limiting the possibilities associated with such membrane changes to recall some elementary facts concerning the relation between membrane polarization and mechanical state of muscle

* It may be mentioned that under massive excitation there should be another type of "local" current. Just after massive stimulation, we presume that the whole of the cathodal side of a fiber is excited, but the opposite side is not. Hence, there should appear a circumferential propagation of the action potential around the fiber, and in association with this an external circumferential local current continuous with an internal portion that passes transversely through the fiber. However, the evidence discussed above and some to be mentioned later eliminates any such transverse current as a factor determining the rôle of excitation in E-C coupling. It is of interest, also, that this kind of propagation introduces a delay in the excitation of the anodal side of a fiber relative to the cathodal. Assuming that conditions are such that this conduction occurs on each side of a 100 μ fiber through a distance of one-quarter of the circumference, and that the velocity of the wave (at 13° C.) is approximately 1000 μ msec.$^{-1}$, then the delay is about 0.08 msec. We have neglected this very small time in all of our kinetic considerations.

fibers. It is well known that contractile responses occur only in response to depolarization of the membrane. This has already been mentioned for overall contractures; and of course it holds for ordinary contractions evoked by a stimulus applied through a pair of electrodes placed on the muscle, since the wave of contraction is initiated at the cathode and not at the anode where, in fact, the resting polarization is transiently increased. Similarly, if a strong enough electric pulse is passed through a muscle made inexcitable by K^+ or novocaine, a local contractile change develops in the region of the cathode and not of the anode.[47] Furthermore, any mechanical change induced by a depolarization can be reversed by repolarization, even when conditions are set for a contraction if the repolarizing pulse is applied quickly enough. Hence, all these facts prove that it is the depolarizing action accompanying the *rise* of the spike potential that is the essential stimulus which sets in motion the E-C coupling mechanism. If the events connected with the waning of the spike (for example, the outflow of K ions[25, 27]) play any rôle in the twitch contraction, this must be to start some process that brings about relaxation of the muscle. But this is purely speculative, for it is also conceivable that the active state of the contractile material may itself start some side reaction which induces its own relaxation, or it may be self-limiting and thus spontaneously undergo relaxation.

Now, Nastuk and Hodgkin[58] found in their muscle fibers that the action potential had an average peak value of about 120 mV., i.e., the membrane potential changed from a resting value of —86 mV. to an active value of +34 mV. (the signs indicating the polarization of the internal surface relative to the external). There seem to be two possible mechanisms by which this change of potential might act. In the first, we note that in the resting fiber the contractile material is charged and surrounded by an atmosphere of its associated ions, and this whole complex is in equilibrium with the potential at the inner boundary of the membrane. At the peak of the action potential the inner membrane-surface goes positive by 120 mV., and although this change is not very great, it is oppositely directed to the resting state, and the close proximity of the myofibrillae to the membrane means that this small change would cause a tremendous alteration in strength of electric field in the critical region. This might then serve to disturb the electrical charge of the immediately neighboring contractile protein and, by setting up an inward-going wave of electrical change, thus constitute the basis of the spike-activation link. In the second possibility, we consider the changes across the membrane as of primary significance. If the membrane is assumed to be 10^{-6} cm. thick, then its change of potential in the transition from rest to peak activity would constitute a reversed field strength of 1.2×10^5 V.cm.$^{-1}$. It is conceivable that such an intense alteration, oppositely directed to the normal field and established very suddenly, could greatly disturb the molecular organization of the membrane and thus possibly cause the release of some membrane component which would then

determine activation. Although both of these mechanisms might operate together, evidence will be described later indicating that at least the second one really exists. At any rate, the proposed mechanisms clearly suggest how the active depolarization of the membrane sets up powerful electrical forces which could conceivably lead to activation of the myofibrillar substance.

The foregoing discussion is based on the view that the purely electrical aspect of the rise of the spike potential is of importance. But much recent work on nerve axons[25, 27–29] has established that this phase of the action potential is caused specifically by the movement of Na ions inward across the membrane; and the general similarity of excitatory events in muscle fibers, in so far as they have been studied,[58] permits us to assume that this Na^+ inflow occurs in these fibers as well. It is therefore of interest to enquire whether the entry of Na^+ might act as a specific chemical stimulus in the activation of contraction.

It is known[26] that the Na^+ entry during active depolarization of invertebrate axons is about 4×10^{-12} mol. cm.$^{-2}$ impulse^{-1}, and Nastuk and Hodgkin[58] suggest that this flux is approximately twice as great in frog sartorius fibers. If we assume this, and further assume that the fiber diameter is 10^{-2} cm. and that due to electrical restrictions at the membrane the penetrating sodium ions are limited to a layer only 10^{-7} cm. thick just inside the membrane, then it can be calculated that the increase in concentration of Na ions in this layer will be of the order of 0.08 M impulse^{-1}. Since the normal Na^+ molarity inside the fiber is about 0.02, this amount of suddenly introduced extra Na^+ would cause a relatively large local increase in concentration of this ion that might serve to set off the spike-activation link by means of some specific chemical reaction with a component of the myoplasm. But experiments[28] have been done in which relatively large volumes of 0.123 M NaCl have been injected into small lengths of isolated, living muscle fibers and no mechanical effect was observed, even though similar injections of only 0.002 M $CaCl_2$ caused a considerable shortening. Furthermore, depolarization of a muscle by immersion in isosmotic KCl causes a contracture even though no external Na^+ is present, and therefore no penetration of Na^+ could possibly occur. In view of this evidence, we may conclude that the inflow of Na ions during excitation is of no consequence in setting up a new chemical milieu which would initiate the S-A link. This all the more stresses the point that it is the spike-*potential,* as such, that performs this critical function.

Spike potential safety factor

One of the many questions that have arisen in discussions of E-C coupling is concerned with the relation between the magnitude of spike potential and accompanying mechanical output. Fulton[19] presents the view that "the electric response is produced by the same mechanism that controls

the initial energy liberation in muscle," and that these two responses therefore vary in parallel. Although they have based their views on different kinds of evidence, Brown[4] and Fleckenstein[16] have proposed a similar type of proportional variation. Contrary evidence, however, has appeared in various pieces of research[7-9, 40, 66, 70, 71] which proves that under certain conditions muscles show variations in tension output which bear no fixed relation to the size of the action potential.

A summary of some of our own results pertinent to this point is presented in Figure 3. Each double bar shows average values, relative to the normal, of the action potential and the simultaneously obtained maximal isometric

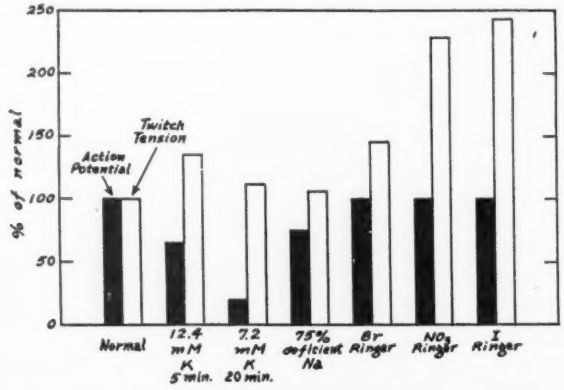

FIG. 3. A comparison of the variations in action potential and twitch tension developed in frog sartorius muscles following various treatments. In each of the last four treatments the relevant muscles were equilibrated for 30 min. to each of the indicated media. Na deficiency was compensated by replacement with choline. In each of the anionically modified Ringer's solutions, the indicated anion completely replaced the normally present chloride.

twitch tension of frog sartorius muscles subjected to a particular treatment. Action potentials were recorded diphasically by external electrodes, and the peak of the first limb was used as a measure of the spike potential. (The general procedure otherwise is obvious from the details of the figure, but further information will be found in various papers.[40, 68, 70]) It might be said that this technique would not yield results indicative of the true relation between spike potential and twitch tension, since the action potentials were not recorded with an internal electrode and there is no exact measure of the spike, as against the entire action potential. There is some validity to this criticism, but, generally speaking, external electrode measurements of the action potential are adequate for approximate determinations of its relative height and so of its spike. And this is shown, e.g., by the fact that in 75% deficient Na^+ media we obtain an action potential that is 75% of

normal, which is not far from the 70% value found for this condition by Nastuk and Hodgkin. Hence we believe that the results shown in Figure 3 are at least an approximation of the true state of affairs.

The evidence given in the figure proves that there is a considerable lack of dependence of the intensity of the contractile response on the potential of the spike: it will be noted that in the presence of enhanced K^+ and of deficient Na^+ the tension output is potentiated above normal even though the action potential is depressed, and that after equilibration to anionically modified Ringer's solution there is a marked mechanical enhancement in association with normal sized action potentials.

These results demonstrate that the spike potential serves as a true trigger in the initiation of E-C coupling. The essence of a trigger is that the intensity of its action, provided it is at least of threshold level, bears no necessary quantitative relation to the magnitude of the change it sets off; i.e., it operates in general with a definite safety factor. This kind of mechanism is already well known for other physiological processes, for example, in connection with the rôle of the safety factor in conduction of the action potential[42] and in junctional transmission relative to the operation of the end-plate potential in exciting the muscle fiber.[48] Thus, to these cases of physiological triggers we can now add the function of the spike in E-C coupling. Although our present results set no clearly defined limit to the value of the safety factor of this trigger, it would seem to be of the order of four to five at least.

From our previous discussion of the sequence of reactions of E-C coupling and of the rôle played by the spike potential, it would seem that the spike acts as a trigger directly on the spike-activation link, and that consequently the processes of E-C coupling automatically proceed, with each reaction determining the next, until contraction finally ensues. We will have more to say about the spike-activation link in this sequence later. But it is of interest to note now that the electrical fields set up by the spike, at or across the membrane, are so intense that they could possibly be more than powerful enough to activate this link, and this might be the explanation of the presence of the safety factor.

The demonstration that the spike acts as a trigger with a rather large safety factor raises a number of interesting problems. One is the mechanism by which a prolonged depolarization causes a contracture: does a safety factor operate in these responses? This seems to be excluded by results[18] which show that contracture shortening of frog gastrocnemii is proportional to the depolarization caused by a wide variety of agents. It is therefore suggested that the trigger function holds only if the potential change occurs with the great rapidity of the spike. Another problem is the clarification of the kind of coupling between membrane and contractile events that occurs in the unpropagated responses that are set up by very strong electric shocks in muscles made inexcitable, e.g., by K^+ or procaine. Does the shock under

these conditions evoke a local, triggering change in membrane potential, or does it act directly on the contractile material?

Finally, it is interesting to consider Fleckenstein's theory[15]—that energy for contraction is obtained directly from the energy of the action potential—in relation to the trigger function of the spike. This theory is certainly in contradiction to the presence of the spike's safety factor, but it can also be shown to be incorrect on other grounds. No extended critique will be attempted here: however, it will be shown that in a maximally contracting muscle the energy of the action potential, even when maximal in each fiber, is far less than that of the twitch as a whole. Hodgkin[20] has determined that in association with the action potential in a single *Loligo* axon of 500 μ diameter the electrical energy liberated is 24 erg gm.$^{-1}$ impulse^{-1}. This was done by use of the formula that the energy, $W = CV^2$ where $C = 1.5$ μf for each cm.^{2} of membrane, and the peak spike potential, $V = 0.1$ V. In the frog muscle fiber $C = 5$ μf cm.$^{-2}$ (Katz[43]) and $V = 0.120$ V. Furthermore, these fibers have a diameter of about 100 μ, and thus it can be calculated that the total membrane area of 1 gm. of fiber is 5 times that of the *Loligo* axon (this being important in connection with the total capacitance of the fiber). Taking into account these differences in the electric constants and the geometry of the fibers, it turns out that the energy of the action potential of muscle fiber is 570 ergs gm.$^{-1}$ impulse^{-1} or 1.3×10^{-5} gm. cal. gm.$^{-1}$ impulse^{-1}. This energy release will be less for the total length of the fibers in one gram of intact muscle, since the fibers make up only about 0.85 of the mass of the muscle. Thus, the actual value will be 1.1×10^{-5} gm. cal. gm.$^{-1}$ impulse^{-1}. We may now compare this energy of excitation with the energy of a twitch. Hill[28] has determined that in a maximal isotonic response the total release (activation heat + shortening heat + work) can be as high as 2.8×10^{-3} gm. cal. gm.$^{-1}$ twitch^{-1}. Since this is set free in response to only a single impulse in the fibers, it is evident that under normal conditions the energy of the action potential is only about 0.4% of that released in the contraction; and in view of our previous discussion of the safety factor, this may be as low as 0.1%. For this reason, it is evident that Fleckenstein's theory is not acceptable. Furthermore, our calculation has added significance with regard to the trigger action of the spike, for it demonstrates how small is the energy expenditure of the excitation process in comparison with the energy set free in the contraction.

The spike-activation link

Of all the mysteries of E-C coupling, there is no doubt that the spike-activation link is the most mysterious; moreover, because it is critically important as the mechanism that couples membrane events to the earliest activity of the contractile substance, it is also the most provocative. We have at present only indirect indications of the nature of this process, part of which is purely kinetic. Our previous discussion shows that at 13° C. an

interval of 1.2 msec. elapses between the crest of the action potential and the onset of the latency relaxation, and we ascribe this duration to the operation of the S-A link. We have made some preliminary analyses at other temperatures with results, given in Table 1, that are so definite that they are worth noting. It is clear that over the range from 6.6° to 25° the time taken up by the S-A link is fairly constant, even though the durations of the rise of the spike and of both the intervals L_R and L_1 vary widely. This indicates that this reaction has a temperature coefficient of the order of 1, which suggests that the underlying process is fundamentally physical, as it must be if the electrical forces of the membranes previously discussed are responsible for its initiation.

TABLE 1

The effect of temperature on the duration of the spike-activation link. The values (rounded) for the spike-rise at 6.6 and 13.0° C. have been obtained directly from Nastuk and Hodgkin,[58] and the value at 25.0° C. has been calculated from their data by using a $Q_{10} = 3$. The L_R and L_1 durations are average values obtained from our own observations.

Temperature, ° C.	*Time interval, msec.*			
	Rise of spike	L_R	S-A *link*	L_1
6.6	1.5	2.5	1.0	8.8
13.0	0.6	1.8	1.2	5.4
25.0	0.2	1.0	0.8	2.5

However, our S-A link interval is determined in such a way (not only by the value of L_R, but also by Hill's determinations of the earliest appearance of both the activation heat[29] and the effects of quick stretch[32]) that it measures the time required to pass on the effects of excitation to the contractile components which are the very first to show activation; and these elements are probably the ones that lie nearest to the surface since they would immediately be affected by the excitation changes taking place in the adjacent membrane. Hill has also obtained results for frog sartorii at 0° C. which give a measure of the time that it takes for achieving the state of full activity. From his activation heat studies,[27] this appears to be about 30 msec., and from quick stretch experiments,[28] 40 msec. Now the mean value of L_R for the frog sartorius at 0° C. is 7.0 msec.[3] Hence the average time needed to complete activation of all the contractile components of a fiber is about 28 msec. This is evidently the time required for the wave of activation to be propagated from the most superficially placed myofibrils to those at the center of the fiber, i.e., for a 100 μ fiber, through a distance of 50 μ (since the wave starts at the circumference and moves inward toward the center). Hence, at 0° C., the over-all effects of the inwardly going wave of activation suggest that its apparent velocity is 1.8×10^{-4} cm. msec.$^{-1}$.

Results of Abbott and Ritchie[2] indicate that the corresponding propagated action potential has a speed of 560×10^{-4} cm. msec.$^{-1}$, so that the wave of activation is slower by a factor of several hundred.

Nonetheless, in another and very important context, this wave of activation is very rapid. The various papers of Hill show that it brings about the state of full activity within only the first tenth of the usually recorded contraction period of a twitch. Taking into account the kinetics of diffusion processes, he has shown by a convincing theoretical analysis that "it is quite impossible, therefore, to explain the rapid development of full activity in a twitch by assuming that it is set up by the arrival at any point of some substance diffusing from the surface; diffusion is far too slow." Consequently, it is likely that "we must look for some physical or physico-chemical process which is released by excitation at the surface and then propagated inwards."[28] (See also [44].) Nevertheless, it should be noted that a substance liberated at the membrane may be effectively transported to the center of the fiber by a kind of exchange diffusion.[28] That is, if some substance (e.g., Ca ion) is ejected from the membrane as a result of excitation, it could form some complex with the contractile material of the nearby myofibrils, thus causing activation. We might then suppose that dissociation of the complex occurs and the activating substance becomes free to act on the next deeper myofibrils. Thus by a repetition of these processes each element of contractile material would be activated and at the same time serve as a carrier in the exchange diffusion mechanism. Transport of an activating substance in this way might be more rapid than ordinary diffusion, for the jumps from one point to the next would be very short and furthermore electrostatic attractions might be involved in the over-all driving force. Thus the mechanism would be able to satisfy the requirement, stressed by Hill, of the high rate of propagation of activation.

In this connection, our calculation of the velocity of the propagation may be of significance in determining the nature of this process. And it may be of interest that, in some unpublished preliminary studies we have made, the results suggest that the rate of propagation of activation has a rather large temperature coefficient (note values of L_1 in Table 1), which is in contrast with the previously mentioned result that the spike-activation link in the region near the membrane is not affected by temperature, at least in the range from 6.6 to 25° C. This result, at any rate, would be consistent with a general conclusion that develops from the previous discussion, that the entire process of activation consists first of S-A link, which occurs just inside the membrane, and then an inward-going wave of activation, which has different characteristics from the initial link and which initiates the response of the whole mass of contractile material in a given longitudinal segment of fiber.

We return now to a special point concerning the S-A link. In the previous section it was indicated that this link might be thrown into action during

excitation by the change of potential at the inner surface of the membrane or by the transmembrane electric field. Evidence for the latter mechanism may be found in some of our results of the effect of excess K^+ on muscle responses,[70] a summary of which is presented in Figure 4. The significant feature of these results is that increases in depth of the latency relaxation and the tension developed in maximal isometric twitches appear in the presence of extra K^+ under such conditions that we know that the added K^+ had not penetrated the fibers. Thus, marked changes in the behavior of the contractile material can occur even though an agent, here K, acts only at the external surface of the fibers, i.e., on the excitatory membrane. Since our results show that the depth of the latency relaxation is especially

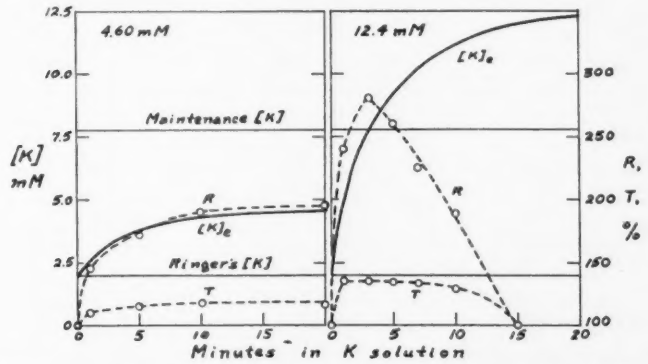

FIG. 4. The effects of Ringer's solutions containing enhanced concentrations of K ions (4.60 and 12.4 mM) on the depth of the latency relaxation (R) and the peak tension (T) (dashed lines) of isometric twitches of frog sartorius muscles. The lines marked "Ringer's [K]" indicate the concentration of K (2.0 mM) in normal Ringer's solution and hence in the extracellular space of muscles equilibrated to this medium. The lines marked "maintenance [K]" indicate the potassium concentration in the extracellular space that must be exceeded before any K penetrates into the fibers. The solid-line curves present the results of a diffusion analysis of the increase of the average concentration of K, $[K]_e$, in the extracellular space following the moment of immersion (zero of the time axis) of the test muscles in their respective media. Note that R and T are potentiated, in either medium, even though $[K]_e$ has not risen enough to cause penetration into the fibers. For further details, see Sandow and Kahn.[70]

enhanced, it is suggested that there is a corresponding intensification of the activation process, at least at the time of the LR, i.e., at the time that the wave of activation is beginning to be set up in the region near the membrane. And we then infer that this potentiation of activation determines the enhancement of the ensuing contraction. But the main question is to explain how the K^+-induced effect on the membrane is transmitted to the contractile material. This could not be accomplished by any electromotive effect of K^+ for, as is well known and as previously shown in Figure 3, K^+ reduces the spike potential and this could hardly be associated with the

accompanying intensification of the responses (both R and T) of the contractile material. And, anyway, our discussion of the safety factor of the spike shows that this trigger need only attain a certain relatively low threshold value to set off the S-A link. We therefore suggest that the K^+ affects some component of the membrane which is not involved in the mechanism generating the spike potential, but which under the influence of this potential is stimulated to develop the S-A link. In the presence of extra K^+ we assume that this component becomes more sensitive to the action of the spike and thus releases a supernormal activation of the contractile response.

The assumption of this membrane component for special service in the S-A link entails the view that the membrane is much more complex than is indicated by its function merely in excitation. But this presents no difficulty, since the known composition of the membrane would seem to be great enough to embrace separate elements for these two processes. Probably the relatively high membrane capacitance of the muscle fiber,[43] which is of the order of five times that of most nerve fibers, reflects the presence of the S-A link component. In any case, our analysis indicates that the triggering action of the spike is exerted at least in part on this special structure within the membrane. Such an action might be expected, for there are many conceivable mechanisms by which various components, oriented or composed in a certain way within the resting membrane, would be violently affected when the intense, reversed field is suddenly set up during excitation right across the membrane. (See, for example, Tobias[75] for a very interesting discussion of such general possibilities in the nerve fiber membrane.)

In connection with the elucidation of the specific nature of the effect of K^+ on the S-A link, it is of interest that K^+-treated cells release Ca^{++} from their membrane and cortical regions into the cytoplasm,[11, 55] and that a liberation of Ca^{++} has been reported upon electrical excitation of normal muscle fibers.[77] Indeed, Heilbrunn[29] stresses that any stimulating agent causes a release of Ca into the cytoplasm and he has proposed on this basis a general theory of stimulation. Now, in our experiments the external concentration of K^+ was much less than that used, in the above cited studies, to cause a release of Ca^{++} into the cell interior. It is therefore doubtful that our K^+-treated muscle fibers at rest suffered any liberation of Ca^{++}. But it is possible that, upon excitation, the muscle fibers in the presence of extra K^+ set free a greater than normal amount of Ca^{++}. Reason in support of this inference is found in the antagonism between K^+ and Ca^{++} effects on the cell surface: K^+ causes a dispersive effect, while Ca does the opposite.[34, 74, 75] Thus, the state of the cell surface is sensitively dependent on the K/Ca ratio in the external medium. Very pertinent to our immediate analysis, for example, are results which prove that the effects of K^+ on muscle we are discussing can be suppressed by a proper increase of Ca^{++} in the medium.[71] It is therefore reasonable to infer that in our treated

muscles the extra K^+ causes a slight "loosening" of the membrane, so that when excitation occurs, the sensitized membrane discharges more than the usual amount of Ca^{++}.

These speculations concerning the behavior of Ca^{++} in relation to membrane states now become of great interest when it is recalled that this ion has pronounced contractile effects on muscle systems: in quite small concentrations it causes shortening either when injected (0.002 M) into living muscle fibers,[93] or allowed to diffuse into them,[77] or when applied (0.001 M or less) to glycerol extracted fibers.[10] Thus, in the normal fiber, the assumed release of Ca^{++} from the extracted membrane might act to engender activation of the contractile system; i.e., this process would serve as the S-A link. Although this would account for the relation between the spike and the activation of the myofibrillae close to the membrane, there yet remains the question of the propagation of activation. This could be effected by the exchange diffusion of Ca^{++} previously discussed, and thus the direct activation of all the contractile material within any fiber would be accounted for by a single mechanism. According to this hypothesis, the intensity of activation would be determined at least by the amount of Ca^{++} released by the trigger action of the spike; and, therefore, in the muscles exposed to extra K, the enhanced Ca^{++}-liberation would explain the potentiated mechanical activity we have observed.

We are fully aware of the highly speculative nature of this hypothesis of activation and of the many rather special assumptions that have had to be made to form its basis. Nonetheless, we are tempted to carry this speculation one step further in order to try to account for the way that Ca^{++} might cause activation. It is well known that Ca is a powerful activator of the myosin-ATPase system; and it is generally believed, though there is great difference of opinion concerning the actual details, that this enzyme system plays a key rôle in the mechano-chemistry of the contractile response of living muscle.[52, 66] We therefore suggest that, in the living muscle, activation of the contractile material (in the sense of Hill) may be attributed to the enzymatic activation of the myosin-ATPase system by Ca^{++}.

Conclusion

A summary of the scheme of E-C coupling that emerges from our study is given in Figure 5. Only the main points have been chosen for inclusion in this diagram, and these, it should be noted, are based on a foundation of facts and quite firm inferences drawn from the facts. The diagram depicts in a general fashion the E-C coupling sequence as it runs its course at a particular point along the length of a muscle fiber, as the action potential sweeps by in a propagated response, or as it would occur along the entire length of a fiber if stimulation had been effected massively. The horizontal direction represents a time axis, with the zero of time marking the instant at which excitation begins. The vertical direction corresponds to a distance

axis, with the membrane, assumed to be 10^{-6} cm. thick, indicated at the top, and below that a relatively very small part of the inner region of the fiber containing the contractile material.

Before excitation the entire system is at rest; the potential of the membrane is 0.086 V., with the inside surface negatively polarized. When the stimulus acts, excitation occurs and the spike potential quickly develops to its crest, thus setting up a reversed membrane potential of 0.034 V., the inside surface being now positive. Hence, a total difference of potential of 0.120 V. has developed and this corresponds to a reversal of electric field

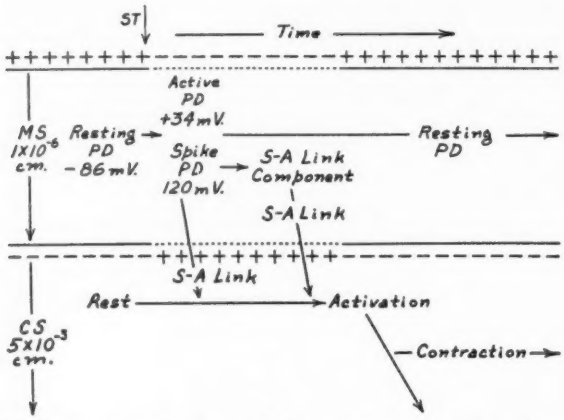

FIG. 5. Diagram of the outline of E-C coupling reactions of the frog sartorius muscle fiber. MS, membrane system; CS, contractile system; ST, stimulus. See text for details.

strength across the membrane of 1.2×10^5 V. cm.$^{-1}$. This change, moreover, occurs very quickly: if we take the case of massive stimulation in which the whole action potential rises to peak in about 0.6 msec., the rate of change of field is astonishingly great, 2×10^8 V. cm.$^{-1}$ sec.$^{-1}$. Our analysis proves that any electric current flow possibly associated with this change plays no rôle in E-C coupling, but that the essential feature is the alteration of potential, and that this is effective certainly across the membrane and possibly also in so far as the introduction of the new charge on the inner surface of the membrane may affect the state of the neighboring myofibrils.

In any case, the spike, in consequence of these potential changes, acts as a trigger to set off the next reaction, the spike-activation link, and it does this with a safety factor of the order of 5 x. The S-A link is probably made up of two mechanisms: one, for which we have some evidence, is a consequence of the spike-transmembrane potential and it involves the liberation

of some substance or agent from the membrane structure itself which then moves into the myoplasm; the other, which seems possible, is due to the direct action of the changed electrical state at the inside of the membrane; this acts rather directly on the neighboring myoplasmic material. In either case the S-A link serves the function of transmitting the effects of excitation to the contractile material just inside the membrane and so causing it to undergo activation thus putting the contractile units into the state through which they must pass in their transition from rest to activity.

Once activation is achieved at the periphery, this initiates a wave of activation that is propagated inward until the whole of the contractile substance is affected. Some preliminary results we present strongly indicate a marked difference between the initial burst of activation involving the S-A link and the later process accompanying the wave of activation, for the rate of action of the S-A link seems to be independent of temperature, while that of the wave of activation is speeded up by the increase of this variable. As soon as any contractile unit completes its activation process, it goes into a state of tension, the active state; and as soon as all the contractile material is so transformed, the full-blown contraction takes place.

This description of E-C coupling is in certain respects only an outline; clearly a formidable array of problems remain yet to be solved. Much more must be learned regarding the membrane component involved in the S-A link. Currently, there are many fascinating approaches to the study of cell membranes, some of which deal with novel permeability,[74, 78] enzymological,[56, 74] and pharmacological properties,[51] and all of which may help to elucidate the initial, surface-mediated reactions of E-C coupling. And Tobias'[78] experiments and speculations concerning the possible effects of electromotive forces on ionic and other changes of membrane and cytoplasmic components may serve to illuminate the problem of the activating effects of the electric fields, set up by excitation, on the S-A link component and on the myofibrils near the membrane.

Whatever may be the mechanism of these processes in normal contractions, we should like to know how they are modified in contractures and in potentiated (or otherwise altered) contractions. In the foregoing we have learned much from considerations of contractures, for they operate under the relatively simple conditions of a sustained depolarization giving rise to a more or less maintained shortening. But we have also pointed out differences between contractures and contractions, and even between contractures of different muscles. There are also the twitch-like contractures evoked by very strong shocks in muscles made inexcitable by various agents (e.g., K^+ or procaine)[9, 13, 64]; probably our recent experiments[60] demonstrating latent addition of the effects of two successive subthreshold shocks in such muscles will advance our knowledge in this field. Nor are these distinctions among contractures of interest only in relation to mechanisms which appear under the rather artificial conditions of experimental alterations of muscle, for, as

shown by the work of Kuffler and co-workers,[49, 50] contractures occur normally in the vertebrate body in consequence of small-fiber innervation of certain muscles, and they are also normal in various motor responses of invertebrates.[44]

As for potentiated contractions, these present many questions. The cases of twitches potentiated by excess in K^+ or deficiency in Na^+ have been previously mentioned; especially interesting are the very much increased twitch tensions produced by muscles in anionically modified Ringer's solution.[40, 41] For in such cases—and there are undoubtedly many more—the substance which affects the response acts only at the surface of the fiber, presumably on the S-A component, and the problem thus arises of accounting for the final enhancement of tension by an effect introduced early in the E-C coupling sequence. Furthermore, this mechanism is of great interest in relation to Hill's results which indicate that even in a twitch the intrinsic tension of the fully activated contractile component is equal to that of a maximal tetanus. It appears that this process of full activation is not fixed in a rigid all-or-none fashion, but that its expression in the actual course of the twitch is amenable to change under the influence of modified E-C coupling reactions. Thus, in the normal muscle, full activation according to Hill might not involve all the contractile units and the unaffected ones might act as a reserve to be called into play in the augmented responses; or activation might be always complete, in which case we would postulate that the E-C coupling sequence under particular conditions sets up changes that could modify the later course of the twitch. Much research is needed to clear up these issues.

The above analysis of E-C coupling is based on evidence of the behavior of striated muscle fibers of the frog; no attempt has been made to explain the events at various neuromuscular junctions, which are inhibitory as well as excitatory. To what extent it holds for other muscle tissue—heart and smooth muscle, or muscles of other organisms, invertebrates, as well as vertebrates—remains to be seen. But we should like to believe that the outline we have presented provides a starting point at least for the study of the corresponding coupling reactions in these other muscles.

As we obtain further details of our E-C coupling sequence, we should also be in a position to describe with increasing certainty the earlier events of contraction itself; for obviously the later events of E-C coupling overlap the earlier ones of the complex of mechanochemical coupling that constitutes the basis for the entire course of a twitch. Thus research on E-C coupling, besides helping to elucidate its immediate problem, has broader implications for the study of the behavior of muscle in general. Especially is this true in regard to the spike-activation link and activation, for these are the key processes that connect the excitation events of the membrane to the initiation of the response of the contractile system. In this connection, our speculations concerning the rôle of Ca^{++} in the detailed operation of E-C

coupling come to mind. Only experimental fact—and that, furthermore, finally obtained in studies on living muscle—can determine whether this hypothesis is correct. But, for the present, we need offer no apology for our speculations, since we value them only in that they enable us to develop a working hypothesis as a basis for further research.

Mysteries abound in the reactions that perform E-C coupling. But they are also found in the attempt to explain any aspect of the fundamental mechanisms of muscular contraction. We hope that our current study has at least defined, to some extent, even if not dispelled, some of the mysteries of E-C coupling, and that it will stimulate further investigations whose results, whatever theory they support, will enable us to advance our understanding of muscular behavior.

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OBSERVATIONS ON THE INFLUENCE OF CORTISONE ON TISSUE RESPONSE TO INJURY*

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Since Hench and Kendall[13, 14] demonstrated the beneficial effect of ACTH and cortisone on rheumatoid arthritis and rheumatic fever, numerous reports on the influence of these drugs on tissue response to various injuries, and on granulation tissue have appeared. Systemically administered cortisone[19–21] and locally administered hog adrenal extract[1] depressed the formation of granulation tissue in wounds in the skin of rats and rabbits. The failure of granulation tissue to form in biopsy wounds[21] and decubitus ulcers[8] in patients being treated with cortisone has also been reported. Heilman[12] has shown that cortisone inhibits the proliferation of fibroblasts in tissue culture. It has been claimed that the administration of cortisone or ACTH depresses the response of connective tissue in healing traumatic wounds in man and in experimental animals.

The influence of cortisone on allergic inflammation appears to be variable and dependent on the dosage, type of allergy, and the animal. Schwartzman, Schneierson, and Soffer[23] demonstrated that cortisone inhibits the Schwartzman phenomenon. Harris and Harris,[11] using large doses of cortisone in the rabbit and guinea pig, showed a suppression of the tuberculin reaction following infection with B.C.G. Sheldon, Cummings, and Evans,[25] using smaller doses of cortisone in the guinea pig, did not demonstrate any suppression of the tuberculin reaction following infection with a virulent strain of *M. tuberculosis*. Cortisone-treated rabbits had little or no antibody response to horse serum, and no Arthus phenomenon could be elicited in them.[10] Dougherty and Schneebeli,[7] using adrenalectomized, cortisone-treated mice did not elicit an Arthus reaction. They also reported a decreased number of polymorphonuclear cells, lymphocytes, and macrophages in the smears of the inflammatory exudate in the cortisone-treated animals. When their results were statistically examined by the writer, it was noted that the control and experimental animals did not differ by more than three times the standard deviation. Schwartzman[24] reported that cortisone-treated mice and hamsters, in contrast to controls, showed a much higher mortality and shorter incubation period following inoculation with a strain of poliomyelitis virus. For some unknown reason, this did not occur when ACTH was used. Cortisone increased the mortality from tuberculosis

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in guinea pigs[4] and rats.[16] Kass, Finland, *et al.*[15] have shown an increased mortality in cortisone-treated mice infected with influenza virus. Cortisone-treated guinea pigs had unusually severe inflammatory reactions when infected with vaccinia virus or various bacteria.[10]

The ability of cortisone to depress antibody formation has been demonstrated.[5, 9, 20] The ability of cortisone-treated animals to cope with bacterial and viral infections is definitely impaired. It is not clear, however, whether this is attributable to a direct effect of cortisone on the cellular and vascular processes in inflammation, or is secondary to the effect on antibody formation, which Rich[23] has shown to be an important feature of resistance to infection. The present report deals with observations on the influence of cortisone on the local inflammatory response to a chemical stimulus and on the cellular, vascular, and histochemical characteristics of the healing phase.

Methods

The present study was conducted on 25 male albino rats weighing 150-200 grams each. They were maintained on Purina lab chow and water *ad libitum*. Lesions were produced by injecting 0.1 cc. Turpentine U.S.P. into the muscles of the front and hind legs and the subcutaneous tissues of the interscapular and lumbar regions.

The experimental group consisted of 15 adrenalectomized rats. These rats were adrenalectomized 48 hours before other experimental procedures were done. During this interval, they were maintained on 0.9% NaCl in place of water. The adrenalectomized animals were divided into the following two groups: 11 rats were started on cortisone acetate 6 to 8 hours before the injection of turpentine, and 4 rats were started on cortisone acetate 24 to 30 hours before the injection of turpentine. They were maintained on 20 mgm./Kgm./24 hours of cortisone acetate (Cortone-Merck) given intraperitoneally in divided doses every 12 hours. An attempt was made to include a third group of adrenalectomized rats which did not receive any cortisone. These animals, however, all died 12 to 15 hours after the injection of turpentine and are not included in this study.

The remaining 10 rats served as controls. A sham operation was performed on these animals. Forty-eight hours later they were given an injection of turpentine. Rats from each group were killed at varying intervals after the turpentine injection to determine the histological picture of the reaction to injury.

Eleven rats were used in the study of the inflammatory lesion. They were killed 24 hours after the turpentine injection. Of these rats, 3 were controls, 4 were adrenalectomized rats that had been started on cortisone 6 to 8 hours before the turpentine injection, and 4 were adrenalectomized rats that had been started on cortisone 24 to 30 hours before the turpentine injection. This last group was included in the study to obviate any objection that an interval of 6 to 8 hours was not long enough to allow the absorption of a sufficient amount of cortisone to exert a maximal effect. The tissues were fixed in 10% formalin buffered at pH 7 and stained by the Maximow hematoxylin-eosin-azure-2 method.

Fourteen rats were used in the study on granulation tissue. Of these, 4 control rats and 4 adrenalectomized rats that had been started on cortisone 6 to 8 hours before turpentine injection were killed 5 days after the injection of turpentine. Three control rats and 3 adrenalectomized rats on whom cortisone was begun 6 to 8 hours before turpentine injection were killed 10 days after the injection of turpentine. The tissues were fixed in 10% formalin buffered at pH 7, and sections were stained with the

Maximow hematoxylin-eosin-azure-2 method, Mallory's trichrome, Laidlaw's silver method for reticulin, and toluidine blue (0.05% aqueous solution at pH 4.5-5.0 for 18 hours). Other portions were fixed in cold 80% alcohol for determination of alkaline phosphatase activity by Gomori's method. These sections were incubated for 24 hours at 37° C. in glycerophosphate at pH 9.0. The relative amount of resulting cobalt sulfide was estimated and recorded.

In the 24-hour lesions, 200 cells were counted and the percentages of polymorphonuclear cells, lymphocytes, mononuclear cells, eosinophiles, mast cells, and fibroblasts were determined. Eosinophiles were recognized by their bilobed nucleus and large red cytoplasmic granules. The counts were made in a radial direction from the center of the inflammatory focus. An estimate of the intensity and extent of the edema was also made.

In the 5- and 10-day lesions the percentages of polymorphonuclear cells, lymphocytes, mononuclear cells, eosinophiles, mast cells, mature and immature fibroblasts in the abscess walls were determined on the hematoxylin-eosin-azure-2 sections. A fibroblast was considered immature if the nucleus was vesicular and large and the cytoplasm abundant, mature if the nucleus was small and more deeply stained and the cytoplasm scanty. These counts were made in one focal plane and in one radius out from the center of the abscess cavity. Each wall was also examined for capillary loops and the percentage of immature fibroblasts. Three separate 0.3 mm. diameters were counted for each abscess and the results averaged. The Mallory, Laidlaw, toluidine blue, and alkaline phosphatase slides were examined for the extent and nature of the connective tissue, reticulin, and the extent and nature of the metachromasia and alkaline phosphatase activity, respectively. Intercellular connective tissue fibers staining black with Laidlaw's silver stain were considered precollagen[6, 17]; those staining brown were considered collagen. Light green, wavy, thin fibers in the Mallory stain were considered immature collagen, while those which were deep green and thick were considered mature collagen. The identity of the slides was not known at the time of making any of the above evaluations.

The means of the percentages of the various cell types and of the number of capillary loops per area were compared in control and experimental animals by the *t* test of Fisher.

Results

Table 1 presents in detail the mean percentages of the various types of cells found 24 hours after the injection of turpentine. No significant differences were noted between the control group and the groups given cortisone either 6 to 8 hours or 24 to 30 hours before turpentine injection. There were slightly significant differences in the percentages of lymphocytes and mononuclear cells between the cortisone sub-groups. Figure 1 graphically presents the distribution of the cell types at varying distances from the center. There is a preponderance of polymorphonuclear cells near the center, but this percentage falls off sharply toward the periphery. No consistent differences were noted in the extent and intensity of the edema, or in the degree of capillary engorgement between the control and experimental animals.

The cell counts in the walls of the abscesses 5 days after the injection of turpentine are presented in detail in Table 2. The cellular nature of the granulation tissue was similar in the experimental and control groups.

Lesions in the cortisone-treated animals showed fewer mature fibroblasts and more polymorphonuclear cells in the central zones than lesions in the controls. There was a tendency for the mononuclear cells to persist at a higher level, for the extent of the wall, in lesions in the cortisone-treated group (Fig. 2). The mean number of capillary loops per 0.3 mm. diameter was significantly lower than the number observed in the control animals.

TABLE 1

PERCENTAGES OF THE VARIOUS TYPES OF CELL 24 HOURS AFTER THE INJECTION OF TURPENTINE

Animal no.	*No. lesions observed*	*No. cells*	*Polymorphonuclear cells*	*Lymphocytes*	*Mononuclear cells*	*Fibroblasts*	*Eosinophiles*	*Mast cells*	
C1	12	2400	49.5	16.3	18.7	11.9	1.0	0.6	Adrenal-
C2	15	3000	63.8	15.6	16.0	3.2	.17	0.3	ectomized
C3	8	1600	54.8	17.7	16.1	9.6	0.4	0.8	plus corti-
C4	16	3200	55.2	19.7	19.7	9.7	1.0	0.7	sone 6-8
Mean			55.8	17.3	16.2	8.6	.64	0.6	hours before
Standard deviation			5.9	1.8	1.9	3.7	.4	.2	turpentine
D1	11	2200	60	12.1	21.5	3.7	0.2	1.6	Adrenal-
D2	14	2800	51.8	11.7	31.8	1.5	2.1	1.1	ectomized
D3	6	1200	47.3	10.6	33.7	4.3	1.9	1.4	plus corti-
D4	10	2000	54.7	10.3	33.5	0	1.3	0.4	sone 24-30
Mean			53.5	11.2	30.1	2.4	1.4	1.1	hours before
Standard deviation			5.3	.86	5.8	1.9	.8	.5	turpentine
A1	13	2600	55.2	21.1	20.3	2.9	.3	.3	
A2	11	2200	63.9	18.0	17.1	.3	0	.7	
A3	18	3600	55.9	20	21.3	1.8	.3	.7	Controls
Mean			58.3	19.7	19.5	1.7	.2	.6	
Standard deviation			4.8	1.6	2.2	1.3	.2	.5	
P values	C-D		>.5	<.01	<.01	>.02	>.1	>.1	
	C-A		>.5	>.1	>.05	>.02	>.1	>.9	
	D-A		>.2	<.01	>.02	.6	>.02	>.2	

The percentage of fibroblasts, in the same diameter, which were immature was approximately the same in the experimental and control animals. These results are presented in Table 3. In the experimental group, practically none of the immature fibroblasts migrated along the capillary loops toward the centers of the abscesses. In the control group, there was a marked migration of immature fibroblasts toward the centers of the abscesses. The connective tissue in the walls of these abscesses was precollagenous in both

TABLE 2

CELL COUNTS IN WALLS OF ABSCESSES 5 DAYS AFTER INJECTION OF TURPENTINE

Animal no.	*No. lesions observed*	*No. cells*	*% Polymorphonuclear cells*	*% Lymphocytes*	*% Mononuclear cells*	*% Mature fibroblasts*	*% Immature fibroblasts*	
M1	7	2667	6.5	3.6	18.7	12.6	64.2	
M2	4	1556	8.5	2.9	21.7	11.5	57.7	
M3	5	1017	6.2	1.8	22.6	16.4	52.4	Control
M4	4	921	11.4	1.9	19.1	8.4	59.1	animals
Mean			8.1	3.3	21.0	12.2	58.4	
Standard deviation			2.4	1.2	1.9	3.3	4.8	
N1	5	1520	14.6	3.3	15.3	4.1	64.5	
N2	8	2823	8.2	5.3	13.9	4.8	67.8	Adrenal-
N3	4	1804	9.6	6.2	12.9	4.4	67.2	ectomized
N4	4	871	12.8	6.2	22.8	9.1	48.7	plus
Mean			11.3	5.3	16.2	5.6	62.1	cortisone
Standard deviation			2.9	1.3	4.5	2.4	9.0	
P values			>.1	>.05	>.05	>.01	>.4	

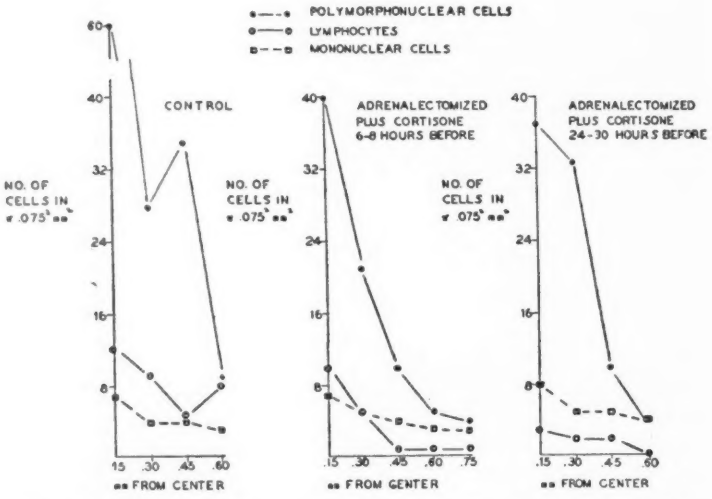

FIG. 1. Distribution of cell types at varying distances from center of lesion 24 hours after injection of turpentine.

groups. Alkaline phosphatase activity was very marked at the centers of the abscesses where there were many polymorphonuclear cells. Peripherally, the alkaline phosphatase activity was not significantly different in either group. It extended throughout the wall. Much of the activity was localized around the fibers of connective tissue. There was less activity noted in the nuclei of the fibroblasts. The polymorphonuclear cells demonstrated marked alkaline phosphatase activity in their nuclei. No metachromatic material was evident, on staining with toluidine blue, in the walls of the

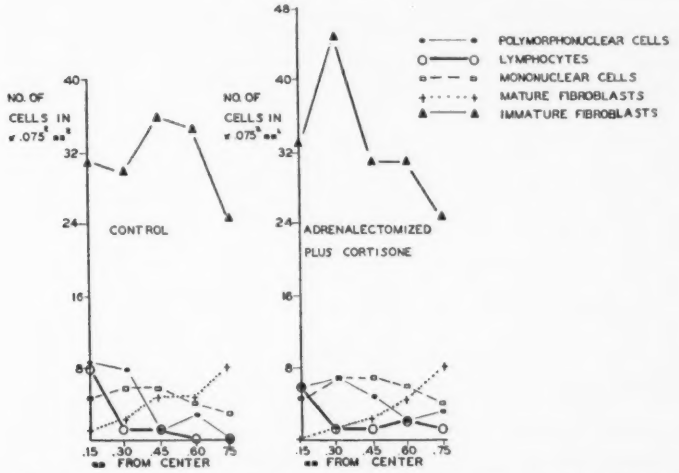

FIG. 2. Distribution of cell types at varying distances from center of lesion 5 days after injection of turpentine.

abscesses in the cortisone-treated animals. In the walls of lesions in the control group metachromatic material was evident. It appeared to be localized, principally about the fibroblasts, and near the capillary loops. The metachromatic material seen in these wounds resembled that found in areas which are known to be rich in mucopolysaccharides.[8]

The results of the cell counts in the 10-day lesions are presented in Table 4. No significant differences were noted in the percentages of polymorphonuclear cells, lymphocytes, and mature fibroblasts. The percentage of mononuclear cells in the cortisone-treated group was significantly higher than in the controls. The lesions of the cortisone-treated animals had a significantly lower number of immature fibroblasts than did the controls. Lesions of the cortisone-treated group had very few mature fibroblasts in the central portions of the walls, but showed a sharp increase in these cells at the periphery. Lesions of the control group had many mature fibroblasts in the

former zones (Fig. 3). The walls of the lesions of the cortisone-treated group were wider than those of the controls, but the number of cells per abscess wall was not greater.

Table 5 presents the data on capillary loops and per cent of immature fibroblasts in the walls of the abscesses 10 days after the injection of turpentine. The number of capillary loops in the lesions of the cortisone-

TABLE 3

PERCENTAGES OF CAPILLARY LOOPS, IMMATURE FIBROBLASTS, AND MIGRATING FIBROBLASTS 5 DAYS AFTER INJECTION OF TURPENTINE

Animal no.	*No. fields observed*	*Capillary** loops*	*% immature* fibroblasts*	*Migrating fibroblasts*
M1	21	6.1	83.5	++
M2	12	7.8	81.4	++
M3	15	7.9	81.0	++ to +++
M4	12	7.4	82.6	++
Mean		7.3	82.1	
Standard deviation		.8	1.1	
N1	15	3.2	75.6	0
N2	24	3.6	79.3	0
N3	12	3.0	80.0	0 to +
N4	12	3.0	90.8	0
Mean		3.2	81.4	
Standard deviation		.3	7.5	
P values		<.01	>.8	

** Counts made with high dry lens; diameter 0.3 mm.

* $\frac{\text{Immature fibroblasts}}{\text{total fibroblasts}} \times 100$; counted in diameter 0.3 mm.

M Control animals
N adrenalectomized animals, plus cortisone
0—none
+—slight
++—marked
+++—very marked

treated animals was significantly depressed. The number of immature fibroblasts in these areas was significantly greater in the lesions in the cortisone-treated animals (Fig. 5).

The connective tissue that constituted the major portion of the walls of the abscesses in the cortisone-treated animals was precollagen; it stained as light green, thin, wavy fibrils in the Mallory preparation, and as black, wavy, argyrophilic fibrils in the Laidlaw stain. There was slight to marked alkaline phosphatase activity throughout the connective tissue of the walls. There was also slight to marked metachromasia in the entire wall. In the

TABLE 4

CELL COUNTS IN LESIONS 10 DAYS AFTER INJECTION OF TURPENTINE

Animal no.	*No. lesions observed*	*No. cells*	*% Polymorphonuclear cells*	*% Lymphocytes*	*% Mononuclear cells*	*% Mature fibroblasts*	*% Immature fibroblasts*	
S1	5	1976	9.1	2.6	9.4	3.6	74.2	
S2	9	3443	10.4	3.4	9.6	4.9	71.5	Control
S3	5	2025	8.8	3.6	8.8	5.6	71.6	
Mean			9.4	3.2	9.3	4.7	72.4	
Standard deviation			.8	.5	.4	1.0	1.5	
R1	5	2464	8.2	6.8	25.3	6.5	49.9	Adrenal-
R2	3	1471	7.2	4.5	24.9	9.0	53.0	ectomized
R3	4	2105	6.3	4.7	21.4	8.7	56.0	plus
Mean			7.2	5.3	23.9	8.7	52.9	cortisone
Standard deviation			.9	1.3	2.1	1.2	3.0	
P values			>.02	>.05	<.01	.02	<.01	

TABLE 5

PERCENTAGE OF CAPILLARY LOOPS AND IMMATURE FIBROBLASTS 10 DAYS AFTER INJECTION OF TURPENTINE

Animal no.	*No. fields observed*	*Capillary** loops*	*% immature* fibroblasts*
S1	15	8.4	45.7
S2	27	9.5	33.2
S3	15	9.0	34.0
Mean		8.0	37.6
Standard deviation		.6	6.9
R1	15	2.7	78.3
R2	9	1.5	75.6
R3	12	2.0	78.8
Mean		2.7	74.2
Standard deviation		.9	4.4
P values		<.01	<.01

** Counts made with high dry lens; diameter 0.3 mm.

* $\frac{\text{Immature fibroblasts}}{\text{total fibroblasts}} \times 100$; counted in diameter 0.3 mm.

S control animals

R adrenalectomized animals, plus cortisone

control group, the outer 40 to 50 per cent of the connective tissue in the walls of the abscesses was mature connective tissue. This portion of the walls stained deep green, with thick strands, in the Mallory stain; and brown fibers were seen in the sections stained with Laidlaw's silver method. This part of the walls demonstrated no alkaline phosphatase activity and no metachromasia. The portions of the walls nearer the centers of the abscesses, in the zone of the capillary loops, showed precollagenous connective tissue,

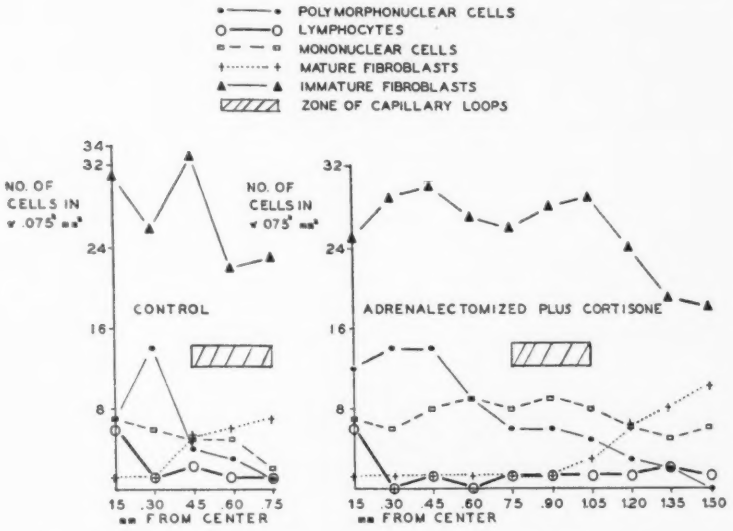

FIG. 3. Distribution of cell types at varying distances from center of lesion 10 days after injection of turpentine.

alkaline phosphatase activity, and metachromasia not unlike that seen in lesions of the cortisone-treated animals (Fig. 6). The intra- and intercellular localization of the alkaline phosphatase activity was similar to that seen in the 5-day lesions. Metachromatically staining material was evident in toluidine blue-stained sections of both control and experimental animals. This material was localized about the fibroblasts and near the capillary loops as in the 5-day lesions.

COMMENT

The failure of cortisone to influence the acute phase of the inflammatory process induced by turpentine is in disagreement with clinical observations on the course of bacterial infection in cortisone-treated patients, and with experiments on bacterial and viral inflammation in animals.[4, 15, 19, 24] These

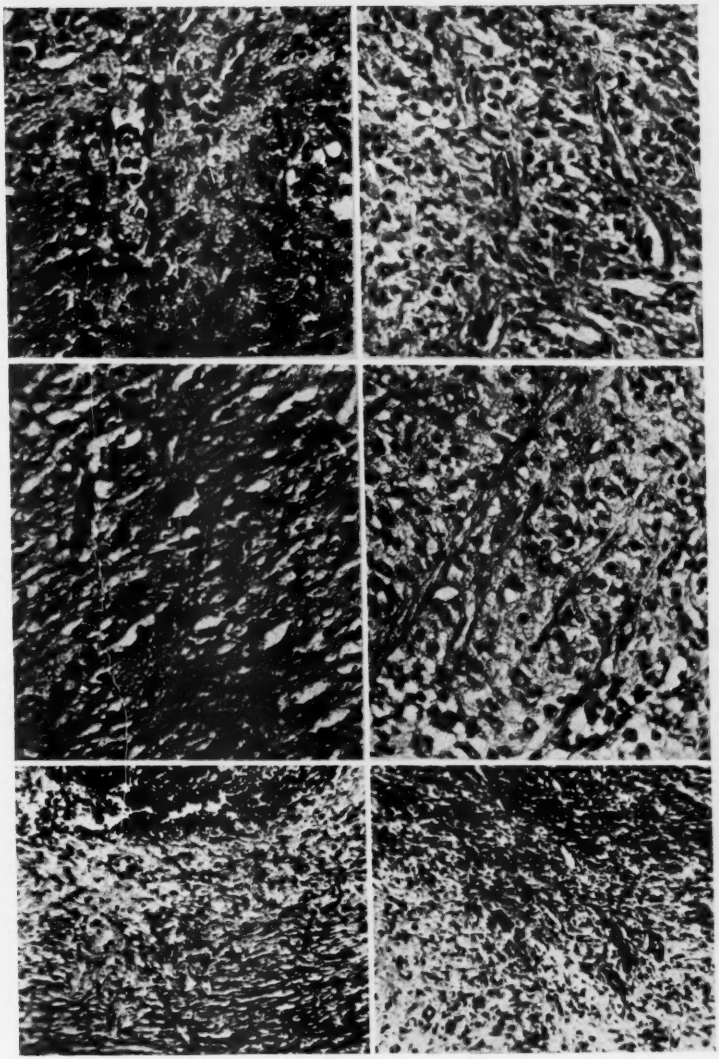

FIG. 4A. Adrenalectomized plus cortisone, 5-day lesion. Hematoxylin-eosin-azure-2 stain. Upper border of photograph is towards center of abscess. Few capillary loops. (x300).

FIG. 4B. Control, 5-day lesion. Same stain as in 4A. Upper border of photograph is towards center of abscess. More capillary loops than in 4A. (x300).

FIG. 5A. Adrenalectomized plus cortisone, 10-day lesion. Hematoxylin-eosin-azure-2 stain. Upper border of photograph is towards center of abscess. Few capillary loops. (x300).

FIG. 5B. Control, 10-day lesion. Same stain as in 5A. Upper border of photograph is towards center of abscess. More capillary loops than in 5A. (x300).

FIG. 6A. Adrenalectomized plus cortisone, 10-day lesion. Upper border of photograph is towards center of abscess. Alkaline phosphatase activity present throughout entire wall of abscess. (x118).

FIG. 6B. Control, 10-day lesion. Upper border of photograph is towards center of abscess. Alkaline phosphatase activity mainly limited to inner portion of abscess wall. (x118).

reactions that are influenced by cortisone are produced by antigenic agents. Rich[22] has shown the importance of the antigen-antibody reaction in inflammation. Some investigators have demonstrated that cortisone depresses antibody formation.[8, 9, 26] From the results of this experiment it appears that cortisone does not influence the processes of exudation, capillary dilatation, or edema formation following injury by a chemical agent. The suppression by cortisone of inflammation attendant upon the introduction of an antigenic substance may be due to its effect on the antigen-antibody reaction, for which, however, evidence is lacking[20]; or it may be due to an alteration in tissue reactivity to the antigenic substance. The latter possibility gains some support from observations on the effects of cortisone on the Schwartzman phenomenon.[23]

In the present experiment the earliest observed effects of cortisone were decreased capillary growth, absence of migrating fibroblasts, and of mucopolysaccharides. Later, mucopolysaccharides appeared in the wounds of cortisone-treated animals, but maturation of fibroblasts did not occur to the same extent as in the controls. Cortisone also depressed proliferation of fibroblasts; and the persistence of mononuclear cells was a feature of these wounds. The over-all effect of cortisone seemed to be a delay in the process of connective tissue repair, rather than a complete suppression.

The earliest observed cellular change in the repair process is proliferation of endothelial cells and fibroblasts. Tissue culture experiments[18] have demonstrated an adverse effect of cortisone on fibroblast proliferation. In their experiments on local application of hog adrenal extract to wounds, Baker and Whitaker[1] have excluded the possibility that this effect is secondary to a systemic catabolism. The possibility that the effect is a localized cellular catabolism is not excluded. It may be that the delay in the appearance of mucopolysaccharides, failure of fibroblast maturation, depression of fibroblast proliferation, and the persistence of mononuclear cells are secondary to the poor vascularization of the regenerating area. These effects may be produced through the adverse influence of poor vascularization on the metabolic activity of these cellular elements.

Summary

Inflammation and repair in response to turpentine have been studied in cortisone-treated adrenalectomized rats and in untreated controls. Lesions were studied at various intervals after the injection of turpentine.

1. At 24 hours there was no significant difference in the percentage of the cell types, in the estimated extent and intensity of edema, or in the degree of capillary engorgement.

2. At 5 days there were no differences noted in the cellular composition of the granulation tissue. Lesions of the cortisone-treated group had fewer capillary loops than the controls. Although migrating fibroblasts and mucopolysaccharides were present in the lesions of the control animals, none

were found in those of the cortisone-treated group. No differences in the extent and intensity of alkaline phosphatase activity in the walls of the lesions were noted between the two groups.

3. At 10 days there were more mononuclear cells, fewer immature fibroblasts and fewer capillary loops in the walls of the lesions of the cortisone-treated group. The percentage of mature fibroblasts was lower in the lesions of cortisone-treated animals. The outer 40 to 50 per cent of the connective tissue in the walls of the abscesses in the control animals was mature collagen. All the connective tissue in the walls of the abscesses of the cortisone-treated animals was precollagen.

In all sections alkaline phosphatase activity was evident: in polymorphonuclear cells, around the connective tissue fibers, and in the nuclei of the fibroblasts. Evidence for the presence of mucopolysaccharides was found about the fibroblasts and near the capillary loops.

It appears that the effect which cortisone has on this type of inflammation is not exerted on the processes of exudation, capillary dilatation, and edema. Cortisone delays the processes of connective tissue repair. It is suggested that this may be principally due to its effect in decreasing capillary growth.

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YALE UNIVERSITY SCHOOL OF MEDICINE

ALUMNI DAY PROGRAM

Monday, February 23, 1953

8:00 A.M. *Surgical Operative Schedule,* Farnam Operating Rooms
Thoracic Surgery, G. E. Lindskog, M.D., and Staff
Neurosurgery, W. J. German, M.D., and Staff
Cardiovascular Surgery, W. W. L. Glenn, M.D.
Orthopedics, N. M. Shutkin, M.D.

10:30 A.M. *Clinicopathological Conference,* Fitkin Amphitheater
Averill A. Liebow, M.D.

11:30 A.M. *Recent Advances in the Management of Infectious Diseases,* Fitkin Amphitheater
Paul B. Beeson, M.D., and Staff

1:00 P.M. *Lunch for Alumni,* Club Room, Sterling Hall of Medicine
Presiding: Vernon W. Lippard, M.D., Dean

3:00-4:00 P.M. *Meeting of Yale Alumni in Medicine,* Gymnasium, Sterling Hall of Medicine
Presiding, Charles L. Larkin, M.D., President, Association of Yale Alumni in Medicine
Admission Policy of the School of Medicine, Thomas R. Forbes, Ph.D., Assistant Dean
Report to the Alumni, Vernon W. Lippard, M.D., Dean

4:00-5:00 P.M. *Departmental Open House*

5:00 P.M. *Social Hour,* Club Room, Sterling Hall of Medicine

LEWIS HILL WEED
1886 - 1952

LEWIS HILL WEED
1886 — 1952

Yale alumni will be grieved to learn of the premature death of Lewis Hill Weed. Dr. Weed was graduated from Yale College in the Class of 1908 with Charles Seymour and John P. Peters and throughout his distinguished career, first as one of Harvey Cushing's fellows in the early days of the Peter Bent Brigham Hospital and later as Professor of Anatomy at the Johns Hopkins Medical School, he was always active as one of Yale's most loyal graduates and as one of the Medical School's most ardent advocates. Fittingly, in 1947 he was chosen a member of the Yale Corporation and for the last five years has served as a Successor Trustee—the only member of the Corporation representing science and medicine.

Dr. Weed assumed many other responsibilities. Since the time of its foundation in 1930 he has been a Trustee of the Institute for Advanced Study at Princeton, and he was also proud of the fact that he was a Trustee of the Carnegie Foundation. Trustee responsibility, he often said, was a matter quite different from faculty responsibility, and he warmly disapproved of faculty members participating and voting in matters that related to trustee function.

Weed was a passionate believer in libraries and in this he had no doubt been influenced by Welch, Osler, Howard Kelly, and his early tour of duty with Harvey Cushing. It is not generally known that while Dean (1923-29) and Director (1929-46) of the Johns Hopkins Medical School he raised the funds, practically singlehanded but with Welch's blessing, for the Welch Library and the Institute of the History of Medicine. He likewise gave us moral support when Dr. Cushing was agitating for a new medical library at Yale, and as a member of the Yale Corporation he gave active support to those who wished to create a department of the history of medicine. He also favored the creation of a history of science library at the Institute for Advanced Study—a library generously donated last year by Mr. Lessing Rosenwald. Ever modest, these things were almost never mentioned, but if the direct question were put, he would no doubt have responded that the creation of libraries and new chairs in any institution of learning is a trustee function, while *appointments* to any chair, once created, is entirely an affair of the faculty.

Weed will be remembered for much else, and especially for the eminent Department of Anatomy which he developed in Baltimore in the best Hopkins tradition. He trained many men who later assumed positions of responsibility, men such as George Wislocki, now Professor of Anatomy at the Harvard Medical School, Louis Flexner, the Professor of Anatomy at

the University of Pennsylvania, Adolph Schultz, the Professor of Physical Anthropology at Zurich, Marion Hines, now Professor in the Department of Anatomy at Emory University—these, and many others. He gave no encouragement to staff members who might wish to stay indefinitely at the Johns Hopkins, pointing out to them in no uncertain terms that they were there for training for the assumption of higher responsibility elsewhere. He was likewise reluctant to recommend salary increases for any of his more able staff members because he felt that this might give them a false sense of security and that they would be disinclined to accept important "calls" to other universities.

Dr. Weed had a very broad horizon as far as the medical sciences were concerned, and it was a surprise to no one when he was called in 1939 to become Chairman of the Division of Medical Sciences of the National Research Council. During peacetime years this was generally a routine responsibility which could be discharged if the Chairman were in Washington once or twice a week. With the war crisis, Weed was obliged to resign from active participation in his work as Chairman of the Department of Anatomy at Johns Hopkins, and to spend full time in Washington until he retired in 1950. His friends well remember that "full time" constituted breakfast in Baltimore at 6:30 a.m., his desk in Washington at 8:30, a long day of heavy duties concerned with the many committees at work under the auspices of the Council and in connection with the necessity for keeping the medical departments of the Armed Forces fully informed, often dinner in Washington and back to Baltimore on a late train, only to be up the next morning for another 6:30 breakfast.

This went on for ten long years, at the end of which his health broke and he was obliged to spend a year at Saranac, New York, under treatment with streptomycin for laryngeal tuberculosis. He responded magnificently and was able to return to some of his trustee responsibilities, but he had meanwhile retired from the National Research Council to be succeeded by Milton C. Winternitz as Chairman of the Medical Division. During the past two years he lived at Reading, Pennsylvania, looking after family affairs and his recently widowed brother-in-law, Dr. Charles Essick, also a graduate of the Johns Hopkins Medical School, who has been an invalid during the past year. Dr. Weed's general health was good until a few months ago; three weeks before his death he had a mild heart attack and he died in his sleep during the morning of 21 December.

In recent years many honors came to Lew Weed, and he received each one with a disarming boyish pride that delighted his friends. For his war service he was given the President's Medal of Merit, and the British Government conferred upon him the Order of the British Empire (O.B.E.). He also received eleven honorary degrees from universities in this country and

abroad. The one which perhaps pleased him most was an LL.D. *honoris causa* from his own Johns Hopkins University on 22 February 1951, on which occasion his devoted colleague George Corner read the following citation:

> This distinguished man, a native of Cleveland, and a bachelor of arts of Yale University, first came to Johns Hopkins as a student of Medicine. Even before he completed his professional studies he began a notable investigation of the structure of the human brain. After a brief post-graduate venture into surgical research at Harvard University under the eminent direction of Harvey Cushing, he returned to the Johns Hopkins anatomical department, rising rapidly in academic rank until in 1919 he became Professor of Anatomy. During these years he continued his studies of cerebral structure. He published a famous investigation of the formation and flow of the cerebrospinal fluid, and skillfully explored the dura mater and pia mater, those membranes that envelope and guard the organ of thought. The scholar who made these studies of the brain, its nuclear masses of nerve cells, its pathways of communication, and its sheltering membranes, was soon called to direct the living brains of physicians and men of science in action for the public good. Wherever he worked there has been a nucleus of scientific thought; he has guided the intercommunications of medical science, and has created shelter and support for research. For many years he was Dean of the Johns Hopkins Medical Faculty and Director of the School of Medicine. The Welch Library, erected under his supervision, is a permanent symbol of his administrative scope.
>
> In 1939, when the entrance of the United States into the Second World War was foreseen, he became Chairman of the Division of Medical Sciences of the National Research Council. Spending his energies and his talent for organization with unselfish disregard for his personal interests, he built the Division into a great agency for public service. During the latter years of the war he was also Chairman of the Medical Advisory Board of the American Red Cross.
>
> Thus, beginning his career in the dissecting room, a seeker of scientific knowledge in the dismembered human frame, this man has become a leader and counselor of those who heal the broken bodies of men, who guard our health in war and disaster and in time of peace unite themselves in constructive research for the welfare of our race.
>
> JOHN F. FULTON

EVENTS

PHYSIOLOGY-PHARMACOLOGY SEMINAR

November 11, 1952

DISTRIBUTION OF SYMPATHIN IN THE CENTRAL NERVOUS SYSTEM. By Marthe Vogt, Department of Pharmacology, Edinburgh University, Edinburgh, Scotland.

The distribution in the central nervous system of sympathin, a mixture mostly of norepinephrine and epinephrine, appears to be correlated with the function of autonomic centers. Sympathin was extracted from various regions of the brain with alcohol, separated by paper chromatography and determined biologically. The hypothalamus and area postrema, especially the former, are rich in sympathin while the mammillary body, midbrain, cerebellum, caudate nucleus, and cortex contain very little. Drugs that are known to cause splanchnic stimulation, such as morphine, picrotoxin, ether, and nicotine, deplete by fifty to sixty per cent the content of sympathin in the hypothalamus.

J. P. G.

YALE MEDICAL SOCIETY

November 12, 1952

REPAIR OF INTERAURICULAR SEPTAL DEFECTS. By William W. L. Glenn.

In these experiments the "open-heart" approach to artificially created interauricular septal defects of dogs was used. The venae cavae and the aorta are cannulated and connected through a pump, respectively, to the femoral vein and aorta of another dog. Fibrillation is then produced by an electric shock, the auricle is opened and the defect repaired with a pericardial graft under direct vision. Next the heart is completely stopped by another shock and then regular sinus-rhythm reëstablished by successive electrical stimuli.

The main problems encountered in the procedure are (i) disposal of the return from the coronary and bronchial circulations, and (ii) prevention of foaming, or the removal of formed bubbles. These are handled as follows: coronary sinus return is removed by periodically suctioning the right ventricle and returning the blood to the circulating volume. Bronchial venous return is permitted to accumulate in the left side of the heart. Foam is removed by circulating the blood through a system filled with glass beads coated with an anti-foaming agent. The drying of exposed endothelial surfaces and trauma due to cannulation leading to thrombosis are hazards which can be minimized by careful technique.

A moving picture was shown, illustrating the major steps of the procedure.

N. A. H.

VIREMIA IN EXPERIMENTAL POLIOMYELITIS. By Dorothy M. Horstmann.

The high incidence of viremia with subsequent development of antibodies found in chimpanzees and Cynomolgus monkeys experimentally infected by a natural route with poliomyelitis virus, points to the possibility that viremia may occur in the human disease. Furthermore, the idea that there is a general, vascular, non-neural phase of the disease, as opposed to strict neurotropism, is strengthened by this finding.

Poliomyelitis virus was fed or inoculated cutaneously into monkeys. Daily blood specimens tested for the presence of poliomyelitis virus showed that animals which eventually exhibited outward manifestations of infection—fever and paralysis—had viremia early in the incubation period followed by a disappearance of viremia and development of antibodies; the viremia often preceded the onset of paralysis by four to six days. Fractional blood analysis indicated that poliomyelitis was not associated with the red blood cells, but was distributed equally in whole blood, serum, and plasma.

Direct inoculation of the virus into the central nervous system through intracerebral inoculation, in an effort to by-pass the vascular phase has not proved successful. Because of either a "spilling over" of the virus into the blood, or the possibility that a small amount of the virus was unwittingly introduced cutaneously during the procedure, four of eight animals so treated showed a viremia in the incubation period and antibody formation almost as readily as those which received the virus orally or cutaneously.

Earlier research involving tests on 111 human poliomyelitis patients revealed viremia in only one child, several hours after the onset of a mild abortive attack. Taken with the experimental findings, this suggests that the time to look for viremia in the human disease is during the incubation period, or the "minor illness," or the first phase of the diphasic course.

G. McK.

ADRENAL STEROID SECRETION IN HUMAN BEINGS STUDIED WITH THE INTRAVENOUS CATHETER. By Philip K. Bondy and J. A. Altrock.

The direct measurement of adrenal steroid secretion in the human subject is an important step in evaluating theories of adrenal function which at present rest on evidence provided by animal experimentation and indirect measurements in the human. A catheter may be passed into the subject's left antecubital vein, through the right atrium into the inferior vena cava and into the left renal vein. Measurement of steroid content in samples proximal and distal to the entrance of the adrenal vein, with determination of renal plasma flow by p-amino hippurate extraction, makes possible the calculation of steroid secreted by the left adrenal. Since the calculated PAH extraction represents renal plasma flow in both kidneys, the calculated 17-hydroxycorticosteroid output actually represents the output of both adrenal glands. The amount of F-like substance in the blood is measured by the phenylhydrazine reaction applied to purified extracts of plasma. This method indicates that: (i) more adrenal steroid is secreted in the resting human than is required to maintain a patient with Addison's disease; (ii) five minutes after the intravenous administration of ACTH a rise in output occurs so that the amount of steroid appearing is comparable to the dose of cortisone used in the treatment of rheumatoid arthritis. It is practically impossible, nevertheless, to obtain consistent results. Several difficulties were encountered: (i) some of the active material may be water soluble and escape detection by the method of chemical analysis used; (ii) the large volume of renal plasma flow in contrast to the small amount of steroid entering the renal vein magnifies the importance of small errors in the estimation of the steroid output; (iii) the subject cannot be considered basal after the trauma (partly psychic) induced by the passage of the catheter; and (iv) the extract position of the catheter tip cannot be determined with accuracy. It is suggested, therefore, that this method has only a very limited application.

O. L. K.

ZOOLOGICAL JOURNAL CLUB

November 19, 1952

STUDIES ON RADIATION PROTECTION. By Alexander Hollaender, Oak Ridge National Laboratory.

These studies were carried out in an effort to discover chemical and physical methods of protecting organisms from the effects of ionizing radiation and to determine the mechanisms by which any protection may be afforded. The assay most frequently used was the survival of micro-organisms, but *Tradescantia* and corn pollen, grasshopper neuroblasts, and embryonic mice also served as experimental material.

The concentration of oxygen in the atmosphere surrounding the cells during irradiation has a marked effect on the extent of the damage observed. When this concentration is reduced to a range between one per cent and ten per cent, the rate of survival of irradiated bacteria is increased, and the amount of damage to pollen chromosomes and embryonic mice is decreased. It appears, though there is no proof as yet, that these effects are due to an increased rate of repair of radiation damage, rather than to a decrease in the initial radiation effect. In this connection it should be noted that high concentrations of oxygen in the absence of irradiation will bring about increased breakage in *Tradescantia* pollen chromosomes. In this case, high humidity also increases the breakage frequency.

The concentration of the other gases studied is relatively unimportant. The largest effect is that of an atmosphere of ninety per cent hydrogen and ten per cent oxygen, in which cells having a hydrogenase system show a somewhat greater sensitivity to radiation.

The LD 50 may be increased by certain reducing compounds, such as 0.04 M sodium hydrosulfite, cysteine, BAL, and even by various alcohols, glycols, and organic acids. These last three classes of compounds are effective only when the organisms are incubated with them prior to irradiation. In no case is any protection afforded by the agent if it is applied after irradiation. The nature of the protection seems to be equivalent to lowering the oxygen concentration, at least in some cases. When metabolism is disturbed, as by cyanide, the protective activity is reduced.

Yeast extract and meat extract contain protective factors, the natures of which are unknown at present. The resistance of mammals to radiation is increased by transplants of living spleen tissue, or by injections of living bone marrow cells.

Low temperatures increase recovery from radiation. At liquid air temperatures there is as little effect as if there were complete lack of oxygen during irradiation. It is found, too, that there is a pronounced effect on the survival rate of micro-organisms exerted by the temperature at which the cultures are incubated after recovery. The maximum rate of recovery is at about 18° C.

It is estimated that eighty per cent of the effect of ionizing radiation can be overcome by the combined effects of low oxygen tension, low temperature, and various chemicals. The other twenty per cent appears to result from the action of the radiation directly on the sensitive cell structures.

R. R. L. G.

YALE MEDICAL SOCIETY

November 24, 1952

THE EFFECTS OF UNDERNUTRITION ON THE COMPOSITION OF THE BODY. By Robert Alexander McCance, F.R.S., Professor of Experimental Medicine, Medical Research Council and Cambridge University, England.

In normal human subjects of different sizes and shapes, the fat component of the body varies, while the extracellular fluid and cell mass remain essentially unchanged. However, subjects from German prison camps, suffering from chronic malnutrition, had the fat content of a thin man, a greatly increased extracellular fluid, and a decreased cell mass. On rehabilitation, these subjects showed a net gain in weight consisting of a gain in fat, a gain in cell mass, and a loss of extracellular fluid.

The overload of extracellular fluid can be explained by a combination of factors: (i) the partial replacement of the space normally occupied by cell mass and fat; (ii) an altered body weight/surface area ratio; (iii) the high salt content of the diet of these subjects; and (iv) fall in serum protein (edema fluid makes up only a small part of the increased fluid volume).

Blood analysis of these subjects indicated a reduced hemoglobin, a normal globulin, a reduced albumin, a low cholesterol, and a low cholinesterase. The last two findings might be related to the low incidence of arteriosclerosis found in these subjects.

The liver function tests of these individuals were within normal limits. Subsequent investigation of the livers of rats and guinea pigs starved for six days indicated that the structure and function were still intact, although the percentage loss of weight of the liver was greater than that of the rest of the body.

G. McK.

MICROBIOLOGY SEMINAR

November 24, 1952

ANALYTICAL STUDIES OF NUCLEIC ACIDS. By Miss Patricia Fitzgerald, Department of Biochemistry, College of Physicians and Surgeons, New York.

Although the work of Doctor Chargaff's laboratory is concerned with nucleic acids in their every aspect, the current report is devoted to studies of desoxyribonucleic acid (DNA) in which the two-fold objective of the investigation is to establish the best methods of preparation and purification.

To ascertain the effect of a procedure on the purity of a compound or the integrity of the molecule, such criteria as viscosity or the quality of the DNA as a transforming factor are useful. By such methods, a pH less than 5 or greater than 11.5 and lyophilization can be shown to disrupt the molecule. Furthermore, the enzymic activity of DHAase must be inhibited, which can be best accomplished by blocking the essential Mg^{++} either with fluoride or citrate or, what is even better, ethylenediaminetetra-acetic acid. For the actual extraction of DNA from Serratia marcescens and Mycobacterium phlei, 10% NaCl was found to be sufficiently gentle.

In testing multiple methods of purification, treatment with Duponal (a detergent) followed by the Sevag method removed all but 10% of the unwanted protein. Electrophoresis plus the Sevag method purified Hemophilus influenza transforming factor to 2½% protein, and with this

preparation it was shown that as little as 0.0004 γ from type b and 0.01 γ from type c are active. To date, gross analyses of DNA indicate a structure comprised of purines, pyrimidines, sugars and phosphate. The composition of 5 γ ± 4% of the DNA from bacterial and animal origin has been sufficiently studied by paper chromatography and analysis of the eluent so that the ratios of adenine to guanine, thiamine to cytosine and purine to pyrimidine have been established. When the patterns of ratios of DNA from five sources are compared, there appears to be a species specificity. After treatment with DNAase these ratios are altered.

In association with these experiments mention should be made of a polyribose PO_4 which has been shown to be antigenic, at least as a haptene. It is attacked by RNAase for which pyrimidine was formerly thought to be essential. Quantitatively there is 1 mole of phosphate for 1 of ribose and there is no nitrogen.

M.-L. JOHNSON

NEUROLOGICAL STUDY UNIT

November 25, 1952

INTERVERTEBRAL DISCS: PAST AND PRESENT. By W. Jason Mixter, Boston, Massachusetts.

The operative procedure for the relief of ruptured intervertebral discs has progressed a long way since Mixter reported in 1934 the surgical repair of seven cases previously diagnosed as enchondroma or unexplained sciatica. The use of contrast media, x-ray spot plates, and an increased knowledge of neuro-anatomy and the innervation of skin have enhanced diagnosis and localization.

However, the effect of a rupture of a disc has not changed over the years. The pressure of the "blow-out" extrudes fragments, sometimes visible by x-ray, that produce pressure on nerve roots, usually of a single nerve; collapse of the disc and abnormal mobility of the intervertebral joint follow. The operative procedure is performed to relieve pain and muscular weakness which are the effects of pressure on nerve roots; but it is imperative to relieve pain without sacrificing the mobility of an affected part, and encroaching on a normal disc and surgically producing a rupture.

The postoperative care and the advisability of simultaneous spinal fusion are still problematic.

G. McK.

NEUROLOGICAL STUDY UNIT

December 8, 1952

ELECTRICAL STIMULATION AND ELECTROLYSIS OF THE HUMAN BRAIN. By J. M. R. Delgado.

Extensive animal experimentation indicated the possibility of implanting permanent, flexible electrodes into the human brain for recording, stimulation, and lysis. Stimulation of the frontal lobes of a monkey (*Macacus rhesus*) produced no emotional disturbances or interference with previous training. However, as demonstrated in a movie, stimulation of the hippocampal area can produce an increase in aggression, stimulation of the thalamus (Nucleus subparafascicularis) can produce an inhibition of aggression, and destruction of the pathways connecting the frontal lobes and the hypothalamus can induce the appearance of catatonic states and sleep.

Stimulation, recording, or destruction is carried out on patients fully recovered from the anesthesia of an operation in which four multiple electrodes are introduced into the frontal lobes through burr holes. The operative procedure is similar to ventriculography, and carries no greater risk. The patients show no sign of discomfort while the electrodes are in place, during stimulation and lysis, or while the electrodes are being removed.

The purpose of the procedure is to study in each patient the areas that are to be destroyed by coagulation. The results obtained from electrical stimulation and electrical recording direct the placement of electrolytic lesions through the same electrodes. As the electrodes remain within the brain for about six days, there is plenty of time to make a careful study of each case and to perform a gradual electrolysis of pathways. A patient suffering from tabes dorsalis was freed from intractable pain by a small lesion produced just under the cortex by this method.

As well as providing a method for producing a localized frontal lobotomy or for stimulation and lysis of the hippocampal region, this procedure also offers an exceptional opportunity to obtain data about the physiology of the human brain.

G. McK.

MICROBIOLOGY SEMINAR

December 8, 1952

ACETOACETATE METABOLISM AND RELATED PROBLEMS. Minor J. Coon, Associate Professor of Biochemistry, University of Pennsylvania, Philadelphia, Pennsylvania.

In recent years isotope studies have confirmed the fact that acetoacetate is synthesized by several pathways, including the condensation of 2 acetyl Co A's. Combination of acetylphosphate, CoA, and a transacetylase will form acetyl Co A which, in the presence of Mg^{++} and an enzyme, at pH 8.1, will go to acetoacetate. Under similar conditions, liver will also synthesize acetoacetate, but heart will not. However, if succinate is added to the heart preparation, some acetoacetate is formed and if succinate plus an enzyme, which has been named deacylase, is added, the yield of acetoacetate is greatly increased.

The four steps postulated for the formation of acetoacetate are:

1. 2 Acetyl Co A → Aceto acetyl Co A + Co A
2. Aceto acetyl Co A + Succinate → Succinyl Co A
3. Succinyl Co A + H_2O → Succinate + Co A + *Acetoacetate*
4. Acetoacetyl Co A + H_2O → *Acetoacetate* + Co A

Experimental evidence from liver and heart studies indicates that step 1 occurs in both liver and heart, 2 and 3 only in heart, and 4 only in liver.

If the heart acetoacetate enzyme system is resolved into two components A and B, and each is added to a mixture of succinate, succinyl Co A and deacylase, it can be shown that together they yield more than five times the acetoacetate produced by either alone. Liver plus enzyme B will provide a comparable amount, but liver and enzyme A will produce only an insignificant quantity. Hence, A and liver, having similar properties, are either identical or serve the same purpose, and it is concluded that heart enzyme A and the liver preparation comprise one enzyme system, while heart enzyme B is a second.

M.-L. JOHNSON

BOOK REVIEWS

A GUIDE TO THE HISTORY OF SCIENCE. By George Sarton. Waltham, Mass., The Chronica Botanica Co.; New York, Stechert-Hafner Inc., 1952. xvii + 316 pp. $7.50.

More than any other living man, Professor Sarton of Harvard has given to scientists the documentation of the humanistic character of their pursuit. No longer should a student of science look at his subject only as a set of facts to be memorized, or a logical structure encompassed in a series of mathematical expressions. When viewed through the eyes of the historian of science, these data and equations become parts of developing chapters in human history.

Professor Sarton has reminded us that in approaching the humanization of science there is a technique to be mastered. The work under review is an introduction to that technique. Its appearance is especially welcome at a time when many scientists not trained in the methods of historical research are beginning to examine the structure of their disciplines from a historical point of view. In George Sarton, the novice will find a stern but sure guide.

The book begins with three introductory essays on Science and Tradition, based on lectures delivered at University College, London, in 1938. Those who have read Professor Sarton's earlier writings, such as the introductory chapters in the separate volumes of his monumental *Introduction to the History of Science,* or his book reviews in *Isis,* will find in these lectures the same breadth of view and the same uncompromising devotion to the principles of critical scholarship. If the three essays are the reader's first meeting with Professor Sarton, the desire to study the earlier works should be a natural consequence.

The second and major part of the book is a superb bibliography for the student who wishes to enter some area of the history of science. The listing of reading matter is not complete, nor did Professor Sarton intend it to be so; as he explains, the bibliography is a selected one based primarily on his own library. However, the variety and number of titles mentioned make the listing a very comprehensive one. It is with a measure of apology to the author that this reviewer, as a Yale biochemist, must record with regret the absence from the bibliography of Russell H. Chittenden's *The Development of Physiological Chemistry in the United States.*

Scientists owe Professor Sarton much for his scholarly work; in giving us a glimpse of the tools of his research, he has placed us further in his debt. This book should be consulted by everyone who wishes to discover for himself that the stuff of science is among the noblest products of the human spirit.

JOSEPH S. FRUTON

THE PRACTICAL SIGNIFICANCE OF MODERN CARDIOLOGICAL INVESTIGATIONS. By T. E. Lowe, W. B. Kay, and H.A. Luke. Melbourne, Melbourne University Press, 1951. 206 pp. 45*s*.

According to the Preface, the subject of this monograph "was that chosen for the Stawell Memorial Prize Essay of the British Medical Association (Victorian Branch) for the year 1949." The authors state further in the Introduction that "our attention in this essay will . . . be focused on those

techniques in use today, either in experimental or clinical cardiology, which are relatively new and of such importance that a thorough understanding of them is essential to the cardiologist."

As an essay, the book warrants praise for its logical organization and clear development. However, it is doubtful that the cardiologist, or anyone, would gather "a thorough understanding" of modern cardiological investigations from this monograph which dismisses many important techniques with catalogue-like brevity. On the contrary, the usefulness of the book would seem to lie in the area of the non-cardiologist who wants an intelligent introduction to the terminology and general scope of cardiovascular studies without a detailed and critical treatise on each of the many methods in use.

Although some of the investigations described will quickly become obsolete, the book should be a valuable addition to any general medical library.

F. D. GRAY, JR.

MICROBIAL GROWTH AND ITS INHIBITION. CROISSANCE MICROBIENNE ET FACTEURS D'INHIBITION. Geneva, World Health Organization, 1952. 285 pp. $3.00.

The "First International Symposium on Chemical Microbiology," organized by the Instituto Superiore di Sanità, was held July 25-30, 1952 in Rome to mark the inauguration of the International Research Centre for Chemical Microbiology. Eighteen of the papers presented on this occasion have been published as No. 10 in the World Health Organization Monograph Series.

The clinical and technological advances ushered in by the antibiotic revolution have largely overshadowed the continuing progress in our basic understanding of the mechanism of action of antimicrobial agents. The screening method for uncovering microbial antagonism has paid high dividends but we have reached the point of ever diminishing returns. Recognition of this fact has necessitated a shift in our attack with the emphasis on the chemical, metabolic, and genetic aspects of bacterial cellular multiplication. To understand how antibiotics act we must learn how bacteria grow, how individuals vary in a bacterial population, what the usual and alternative metabolic pathways are, what nutritive elements are required, what enzyme systems are involved. With increasing knowledge of the chemical nature of the microbial life cycle, introduction of new antagonistic agents will be made on a rational basis by means of metabolic analogues. This monograph indicates a significant trend toward this approach on an international level. The contributions cover a wide range of subjects, including the following: bacterial reaction patterns, nucleic acid metabolism, utilization of folic acid and vitamin B_{12}, enzyme biosynthesis, bacterial lag phases, drug resistance and its genetic analysis, structural and physiological backgrounds of microbial inhibition, and certain aspects of the chemistry as well as fermentation processes and aeration in antibiotic production. The only significant defect is the omission of any consideration of the rôle of host reaction patterns in the analysis of chemical microbiology. Perhaps this important subject will form the basis of a future symposium and monograph.

R. R. WAGNER

LIVER INJURY. Transactions of the Tenth Conference, May 21-22, 1949. F. W. Hoffbauer, Ed. New York, Josiah Macy, Jr., Foundation, 1951. 320 pp. $3.75.

This book is the latest edition of the series which report verbatim the papers presented at the annual meetings of small groups of competent investigators in a variety of medical fields. Informality and theorizing aloud are the keynotes, and account for much of the book's value and great readability. The names of the participants are well known in the field of medicine —F. W. Hoffbauer, Cecil J. Watson, C. H. Best, Paul György, N. H. Knisely, Sheila Sherlock, R. H. Turner, and Ephraim Shorr, to name but a few. The topics presented cover a wide range and include R. H. Turner's discussion of serum proteins and lipids studied by the ultracentrifuge, Sheila Sherlock's "Effect of Insulin on the Liver in the Normal and in the Diabetic," and K. R. Hill's "Liver Disease in Jamaican Children."

Such a compilation is mandatory reading for those working along these particular segments of the research perimeter and is strongly recommended for those doing related work.

H. O. CONN

FACTORS REGULATING BLOOD PRESSURE. Transactions of the Fifth Conference, February 15-16, 1951. B. W. Zweifach and Ephraim Shorr, Eds. New York, Josiah Macy, Jr., Foundation, 1951. 238 pp. $3.75.

In the Introductory Remarks, Dr. Goldblatt, speaking of high blood pressure, states that, in his opinion, "problem number one, still remaining to be solved, deals with the etiology and pathogenesis of the arterial and arteriolar sclerosis that usually accompanies hypertension." The major emphasis of this conference is, accordingly, on the subject of athero- and arteriosclerosis.

All of the Conference participants agree that arteriosclerosis is associated with an alteration of lipid assimilation, transport, metabolism, or excretion. Philosophically, there seems to be some disagreement about whether emphasis should be placed upon transport, i.e., serum lipids, or upon lipid metabolism. Investigative methods which utilize Cohn's fractionation or ultracentrifugation as a means of separating and quantitating lipoproteins (that lipids do not occur in serum as free molecules was agreed by all participants) provide data concerning lipid transport, whereas isotope labelling of dietary lipids provides a fruitful means of studying lipid metabolism.

Major stress in terms of transport was placed on the beta lipoproteins, the cholesterol: phospholipid ratio, and the S_f 10-20 molecules separated by ultracentrifugation. In the study of metabolism, considerable interest was shown in the evidence that cholesterol can be synthesized *in vivo* from a number of precursors, and that it appears to take part in several complex metabolic functions. Dr. Simms' tissue culture studies of lipid substances capable of appearing as visible fat in cells (lipfanogens) as well as their antagonists (antilipfanogens) suggest that both transport mechanisms and lipid metabolism may play some part in the development of arteriosclerosis.

The informal discussion of the Conference provides an excellent means of harmonizing results which seem to vary in relation to the different techniques used, and which often appear contradictory to the medical man who lacks specialized knowledge in the field of lipid biochemistry. The discussion

also serves to emphasize the areas of agreement and disagreement, so that the non-specialized reader can formulate an intelligent impression of the "firmness" of the various current concepts in the field.

In addition to the discussions of the relation between lipids and arteriosclerosis, the Conference gave brief consideration to renal pressor substances and physical factors related to hypertension and arteriosclerosis.

The publication of these transactions is a highly commendable endeavor, and this particular report should be valuable to the expert on hypertension as well as to the interested student.

F. D. GRAY, JR.

CONNECTIVE TISSUES. Transactions of the Second Conference, May 24-25, 1951. Charles Ragan, Ed. New York, Josiah Macy, Jr., Foundation, 1952. 190 pp. $3.50.

Once a year a small group of outstanding investigators representing various fields of medical research are brought together by the Macy Foundation to discuss the subject of connective tissues. The conference was centered about five papers as a nucleus for discussion: Some Functional Considerations of Ground Substance of Connective Tissues, Isidore Gersh; Chemical Morphology of Elastic Fibers, Albert I. Lansing; Pain Mechanisms in Connective Tissues, Janet Travall; Repair Processes in Connective Tissues, Keith R. Porter; Regression of Scar Tissue, Thomas G. Morrione. Dr. Frank Fremont-Smith acted as moderator with W. Paul Holbrook, Charles Ragan, Daniel Murray Angevine, Granville A. Bennett, Edward W. Dempsey, Paul György, Molly Harrower, Colin M. MacLeod, I. Arthur Mirsky, Benjamin Zweifach, A. Colin P. Campbell, W. S. Hartroft, Kenneth R. Hill, B. G. Maegraith, John H. Talbott, and J. A. Wier participating in the discussion. This booklet makes interesting reading for those concerned with connective tissue research. The section on elastic tissue is particularly informative.

W. G. BANFIELD

LABORATORY TECHNIQUE IN BIOLOGY AND MEDICINE. By E. V. Cowdry. Baltimore, Williams and Wilkins Company, 1952. 382 pp. $4.00.

The third edition of Cowdry's *Laboratory technique* brings this well-known and useful book up to date. The format is the same as before but 113 pages of new material have been added. As all users know, it is very much more than simply an alphabetical listing of techniques and terms. Discussion of controversial issues is often included, replete with references to the papers of the protagonists. Historical development of techniques, e.g. electronmicroscopy and phase contrast microscopy, is presented for the orientation of the reader. In describing a highly specialized technique, of what it can be used for and how it is done, a brief account is given and the reader is then referred to the original article. As before, certain sections are written by specialists in the field, and the key references are listed. Among the additions in the new edition are many histochemical procedures, a description of polarigraphic technique (written by C. Carruthers), phase contrast microscopy, a discussion of phosphatases and their demonstration in tumors (by E. W. Dempsey) and the Papanicolaou technique.

HENRY BUNTING

RENAL FUNCTION. Transactions of the Second Conference, October 18-19, 1951. Stanley E. Bradley, Ed. New York, Josiah Macy, Jr., Foundation, 1951. 178 pp. $3.00.

This book is a summation of discussions on the control of renal function by the pituitary and adrenal cortex. The data on these subjects are presented by outstanding authorities and the conclusions drawn from the data are criticized by representatives of many ancillary fields. In this way the perspectives of several scientific disciplines and specialties are brought to bear on renal function. The book is well organized and yet the "give and take" of the discussions is preserved.

The studies of the effects of ACTH and cortisone on renal function will be of particular interest to the clinician. The rôle of the pituitary will be of greater interest to the research worker and advanced student. The bibliographies which are appended to the chapters add greatly to the utility of the book.

R. E. COOKE

PRINCIPLES OF PLANT PHYSIOLOGY. By James Bonner and Arthur W. Galston. San Francisco, W. H. Freeman and Company, 1952. 509 pp. $5.50.

Having had reason lately to browse through a number of plant physiology texts, the reviewer notes that this book is a well-developed example of a style of textbook writing which is encountered more and more. One may recognize, at least among works on plant physiology, two different approaches: one, universal in treatises and advanced texts, introduces and formulates the concepts required in the field, develops them, and cites the experimental evidence for the various theories related to these concepts. Some elementary texts use this plan; the authors apparently hope that the student can be guided through the mass of details and that he will be led to a more searching and thorough attitude towards the subject and its complications. In the other general approach, which is used by the authors of this book, the material is digested, and each topic is developed from one particular point of view, somewhat like a theorem. This treatment intends to impress the student with the basic patterns, upon which he can elaborate. One is reminded of the texts of theoretical physics which lead one along in a conversational style to regions of comparative difficulty, and then exact payment, in the form of time and effort, for the apparent simplicity, by the "problems" at the ends of chapters. Perhaps the references (after each chapter) in this plant physiology text are in this category. The choice of references appeals to this reviewer; because they are practically all books or review articles they should also please the student, who is assumedly pressed for time.

The authors state that this book is a pedagogical effort, designed for a one-semester course, and is not a reference text; bearing this in mind and the point of view adopted in the writing, one feels they have achieved their purpose. The book is a pleasure to read, beautifully laid out, and imaginatively illustrated. Some may laugh at the illustrations, but no one is likely to forget them quickly, nor miss their point. The book impresses one as very much up-to-the minute, tempting one to look for a proportionality between the number of pages devoted to a topic and the number of recent papers on it. Naturally, the biochemical approaches to physiology are stressed. As is always the case when a choice is made of one side of a controversial issue (for example, phloem transport, concerning which the authors

apparently had some qualms) there can be disagreement; since further experience will doubtless settle these questions, there is no point discussing any of them here.

The text is divided into twenty chapters, grouped in three units, on nutrition, metabolism, and growth and development. The first unit treats photosynthesis, mineral and water relations, and translocation. The second part includes chapters on enzymes, carbohydrates, respiration, and other metabolic processes and substances. This is essentially a brief treatment of plant biochemistry, assuming on the student's part a knowledge of elementary chemistry and botany. The last unit, on growth and development, includes lengthy discussions of growth substances and the physiology of reproduction and differentiation. There is also a short section, "Plant and Environment," on the autecological aspects of physiology.

R. R. L. G.

MODERN TRENDS IN PHYSIOLOGY AND BIOCHEMISTRY. E. S. Guzman Barron, Ed. New York, Academic Press, Inc., 1952. xxii + 503 pp. $8.50.

This volume honors the memory of Leonor Michaelis, one of the founders of modern biochemistry. The twenty chapters are based on lectures given during the summer of 1950 at the Marine Biological Laboratory, Woods Hole, Massachusetts. Each of the essays was prepared by a leader in the subject, and consequently the discussion is of high caliber. The volume is highly recommended. A listing of the titles and the authors may be sufficient to indicate the scope of the volume.

The Mechanism of Enzymatic Oxidation-Reductions (E. S. Guzman Barron)

The Identification of Enzyme-Substrate Compounds (Britton Chance)

The Cytoplasm, Its Structure and Rôle in Metabolism, Growth and Differentiation (John Runnström)

Physiology of the Cell Nucleus (Daniel Mazia)

The Physiology of Cell Division (L. V. Heilbrunn)

Molecular Anatomy of the Red Cell Plasma Membrane (Arthur K. Parpart and Robert Ballentine)

The Measurement of Cell Permeability with Particular Reference to the Erythrocyte (M. H. Jacobs)

The Sodium and Potassium Balance of Muscle and Nerve (H. Burr Steinbach)

Mechanism and Properties of Bioelectric-Potentials (Harry Grundfest)

Chemical Mechanisms of Nerve Activity (David Nachmansohn)

Transmission Processes at Nerve-Muscle Junctions (Stephen W. Kuffler)

On Ultrastructure and Function in Nerve. Nonelectrical, Physical Aspects of Excitation and Conduction, as Deduced Primarily from Polarization Experiments (Julian M. Tobias)

Problems in the Comparative Physiology of Nervous Systems (C. Ladd Prosser)

Biochemical Evolution (George Wald)

Thermodynamics and Muscle (A. Szent-Györgyi)

Studies on the Contractile Protein System of Muscle (W. F. H. M. Mommaerts)

On the Mechanism of Drug Action (Otto Loewi)

Some Metal Complexes with Proteins and Other Large Molecules (Irving M. Klotz)

Some Consideration of the Chemical Structure and Biological Activity of Chymotrypsin (Hans Neurath)

The Oxidative Pathways of Carbohydrate Metabolism (E. S. Guzman Barron)

JOSEPH S. FRUTON

SYMPOSIA OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY. Number V. Carbon Dioxide Fixation and Photosynthesis. New York, Academic Press Inc., 1951. 342 pp. $6.80.

The incorporation of carbon is one of the fundamental activities of living systems, providing them with structural material as well as energy sources. Heterotrophic carbon dioxide fixation, which seems to be required by the oxidative mechanism of cells, and photosynthesis, which provides most of the fixed carbon for the earth's biota, are the subjects of the twenty-one papers which comprise this book. The work described is relatively new, and much of it was made possible by developments in biochemical and isotope tracer methods. There seems to be a certain unity of feeling in the book, perhaps because the papers were designed to be read at the symposium. Individually, the papers may be specialized and technical, but taken together, they give a remarkable survey of the subjects.

One cannot use this book as the only basic source of information on photosynthesis, but it can scarcely be omitted as one of the fundamental sources. Together with Rabinowitch's *Photosynthesis and related processes*, the monograph, *Photosynthesis in plants*, of the American Society of Plant Physiologists, and a few review articles (notably that of Whittingham in the *Botanical Review*), it will doubtless constitute the basic literature for some time. Heterotrophic carbon dioxide fixation has not been known or studied as long as photosynthesis and is accordingly somewhat more completely described. While the papers reported were presented in 1950, and the subjects are rapidly expanding, it is not likely that the book will be outdated sooner than is usual for works of this type, because it provides much basic information which can help the student or research worker understand and evaluate new papers.

The distribution of the articles should be mentioned. Four papers are concerned with the existence and kinetics of the enzymatic processes involved in heterotrophic carbon dioxide fixation, and with the nature and significance of the biochemical pathways. Three papers deal with higher plant physiology: one concerns the relation between carbon dioxide and the organic acid content of certain (Crassulacean) plants; the other two describe the physics and physiology of carbon dioxide uptake by leaves.

The theoretical aspects of photosynthesis are considered in eleven papers. The first three concern photochemistry, particularly the photolysis of water by non-biological systems. Three others discuss the "Hill effect," which is the splitting of water and release of oxygen by cell fragments under the influence of light. Two papers describe experiments with carbon 14-labelled carbon dioxide. This isotope is the best tool available for studying the path of carbon in photosynthesis. The last three papers in this group deal with

the physics and physiology of the over-all process—kinetics, side reactions, and the maximum quantum yield.

The three final papers describe refinements in mamometric techniques which are useful in measuring photosynthetic activity, and some of its applications.

R. R. L. G.

LEONARDO DA VINCI ON THE HUMAN BODY. The Anatomical, Physiological, and Embryological Drawings of Leonardo da Vinci with Translations, Emendations and Biographical Introduction by Charles D. O'Malley and J. B. de C. M. Saunders. New York, Henry Schuman, 1952. xxxv + 215 + 21 pp. $25.00.

The authors of this book have appraised once for all the contributions of Leonardo da Vinci to the known facts of human anatomy, revealing his most remarkable coupling of insight and imagination in the pursuit of his scientific investigations. That he was often mistaken is made quite clear. That he approached this particular part of the vast physical world, which came under his scrutiny, with the eye and mind of the artist rather than the scientist is made equally clear to us by the authors, whose dedication to their immense undertaking is Leonardian indeed.

For they have given us a volume in which science and art go hand in hand. Where the celebrated Vinciano's science wanders from the truth and greatness, his epic draftsmanship restores him to a post of even greater magnitude in art itself. Witness the most remarkable anatomical drawing of all time on page 202 of the book, printed again on the back cover of the dust jacket. For sheer beauty of line and form, for the feel of the very cosmos, this drawing, for all its mistakes, takes its place as one of the noblest works of the human hand.

The book is convincing beyond all doubt in its careful and complete analysis of each of the hundreds of drawings and the accompanying notes, in Leonardo's upside down handwriting. These have been translated and printed in italics facing the pages reproducing the drawings with which they appear. The authors' running comment interspersed with the translations indicates precisely wherein Leonardo was relying upon the findings of Galen, Aristotle, Mundinus, and others, where he used the viscera of animals in drawing the human body; in short, why and where he erred. The introduction, which contains a history of anatomical illustration up to about 1475 when the investigations in this book were taking form, an account of his life, an assessment of his anatomical achievements, his plans for the treatise, and the history of the manuscripts, serves as a most satisfactory background for the perusal and study of the over two hundred pages of facsimiles from the originals of the Vincian genius.

Were Leonardo himself to see this volume he would be fascinated beyond all belief at its revelations, and his artistic eye would be filled with wonder at the beauty and exactness of the reproductions. He would marvel at the order that the authors have brought out of the chaos of his own notes. And perhaps he would admit with this reviewer that only Michelangelo himself would have the temerity to criticize and propose to supersede in excellence these researches. Drs. Charles D. O'Malley, J. B. de C. M. Saunders, the authors, and Mr. Henry Schuman and his artist collaborators have produced one of the great books.

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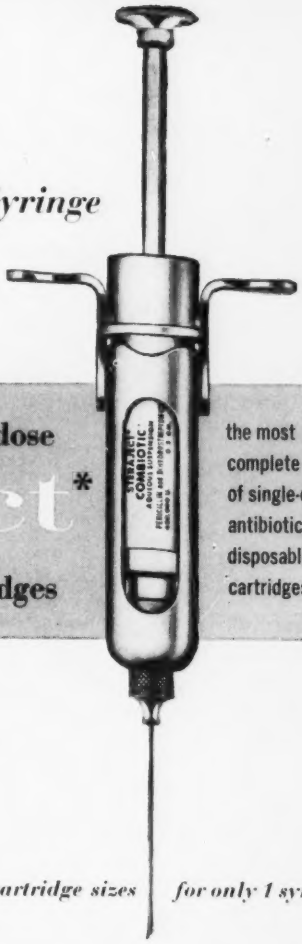

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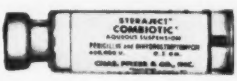

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